Combined Anaerobic-Aerobic Treatment of a Simulated Textile Effluent

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Certificate of Research

This is to certify that, except where specific reference is made, the work described in this thesis is the result of the candidate. Neither this thesis, nor any part of it, has been presented, or is currently submitted, in candidature for any degree at any other University.

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ABSTRACT.

This work aimed to treat a simulated textile effluent (STE) with combined anaerobic-aerobic treatment. A simple simulated cotton processing effluent was generated that was similar to real effluents in terms of COD, BOD, COD:BOD, pH and TSS. An upflow anaerobic sludge blanket reactor (UASB) was more effective in treatment of STE than an inclined tubular digester (ITD) as it treated larger volumes of effluent and showed superior colour removal. Coagulation/flocculation was investigated as a pre-treatment but was found to be unsuitable. It was also aimed to discover whether amines were produced during anaerobic treatment, and if so, were they degraded anaerobically.

Anaerobic treatment reduced the COD load to the aerobic stage and thus reduced the quantity of aerobic sludge produced. However, aerobic treatment alone removed a quantity of COD comparable to that achieved by combined treatment. Unlike anaerobic treatment, aerobic treatment alone did not remove colour. When step increases in load were carried out, or when the UASB was not operating at its optimum, the aerobic stage removed excess anaerobic effluent COD. It was necessary to adjust the activated sludge stage to pH 7 and to feed it a concentrate of OECD synthetic sewage in order to maintain the MLSS. The treatment efficiency of the combined anaerobic-aerobic system remained constant despite intervening step changes indicating that the system could tolerate such changes without any alteration in effectiveness of operation.

The optimum starch:dye ratio for overall colour removal varied with the dye concentration present in the STE. At the same dye concentration the presence of extra starch greatly increased colour removal. It was therefore recommended that if colour removal efficiency decreases, starch to the maximum volumetric loading rate of UASB be added. A combination of HPLC-UV, Total Organic Nitrogen analysis and respiration inhibition tests indicated that anaerobic degradation of the dye used in this STE produced aromatic amines that were removed aerobically. As the amines were degraded aerobically, some aerobic COD removal was due to their degradation.

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ADMI	American Dye Manufacturers Institute
Abs units	Absorbance Units
ASBR	Anaerobic Sequencing Batch Reactor
BA	Bicarbonate Alkalinity
BOD	Biochemical Oxygen Demand
B_v	Volumetric Loading Rate
B_x	Sludge Loading Rate
C.I.	Colour Index
CI	Confidence Interval
COD	Chemical Oxygen Demand
Conc.	Concentration
Cont.	Continued
Cntb.	Contribution
CSTR	Continuously Stirred Tank Reactor
Expt	Small Experiment Within A Larger One
F:M ratio	Food:Micro-Organism Ratio
FID	Flame Ionisation Detector
GAC	Granular Activated Carbon
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IR	Infrared
ITD	Inclined Tubular Digester
LC_{50}	Lethal Concentration To 50% Of The Population
MLSS	Mixed Liquor Suspended Solids
n	Number Of Samples
p	Probability Value
RBC	Rotating Biological Contactor
SD	Standard Deviation
SBR	Sequencing Batch Reactor
SRB	Sulphate-Reducing Bacteria
STE	Simulated Textile Effluent
STP	Standard Temperature And Pressure
STW	Sewage Treatment Works
TOC	Total Organic Carbon
TOD	Total Oxygen Demand
TON	Total Organic Nitrogen Content
TS	Total Solids
TSS	Total Suspended Solids
TVFA	Total Volatile Fatty Acids
UASB	Upflow Anaerobic Sludge Blanket Digester
UV	Ultraviolet
VFA	Volatile Fatty Acids
VS	Volatile Solids

VSS	Volatile Suspended Solids
w/w	Weight Per Weight

CHAPTER ONE - TEXTILE INDUSTRY EFFLUENTS: COMPOSITION AND TREATMENT.

1.1 Introduction.

The textile industry is one of the oldest industries in the world (Trivedy *et al.* 1986). It is among the world's ten largest industrial consumers of water and in Europe has been estimated to account for 2-5% of industrial water consumption (Durig, 1981). In the UK alone, over 60 million m^3 of water are used annually by the textile industry (Berman, 1998). Water consumption for individual processes can range from 3-9 l kg^{-1} for cotton desizing to 334-835 l kg^{-1} for wool washing (Correia *et al.* 1994). Water supply and effluent disposal costs are rising faster than inflation (Rearick *et al.*, 1997) and will continue to do so as water is becoming scarce in relation to demand (Environmental Technology Best Practice Programme, 1997). Pressure is being placed on water companies in Britain by the Environment Agency (EA) to reduce the amount of colour in sewage effluent (Watson, 1991). The combination of high charges for water treatment and supply and new legislation is therefore forcing textile companies to treat their effluents and minimise water usage.

The textile industry is important world-wide and hence its associated pollution has a global impact. Cotton is the principal fibre type used throughout the world (Holme, 1997). It accounts for approximately 50% of the 40 million tonnes annual world fibre consumption (Upton and Churchley, 1997) with values cited ranging from 44.9% to 52% between 1995 and 1997 (Environmental Technology Best Practice Programme, 1997; Holme, 1997; Upton and Churchley, 1997; International Dyer, 1998). It is dyed with reactive dyes, which have a relatively low fixation rate. Therefore effluent resulting from the production of cotton fabrics is significant in terms of pollution. Growth rates for cotton consumption are predicted to be 2-2.5% p.a. (Holme, 1997; Reddig, 1997) which will lead to further associated increases in pollution. Given the increasing popularity of cotton fabrics and the problematic nature of effluent from cotton processing, textile

effluent composition is examined here with particular reference to cotton processing and reactive dyes.

This research was part of a project on "Integrated Water Recycling And Emission Abatement In The Textile Industry" (ENV4-CT95-0064) funded by the European Union under its Environment and Climate Programme. The project aimed to minimise the quantity of clean water required for consumption in the textile industry by developing a treatment process that would clean water sufficiently to allow it to be reused. It also aimed to reduce the quantity of sludge discharged to landfill. Partners included Centro Impresse Depurazione Acqua (CIDA), Como, Italy; Politecnico di Milano (POLIMI), Milan, Italy; Water Research Institute of the Italian National Research Council (IRSA), Bari, Italy; Biotim, Belgium; University of Gent, Belgium; Hoechst Ambiente, (now Aqua Ambiente), Portugal; Instituto Superior Técnico (IST), Lisbon, Portugal; Institut National des Sciences Appliquées de Toulouse (INSA), France; and the University of Glamorgan (UG). Information was exchanged between partners, each of whom concentrated on a different aspect of research into textile effluent treatment. The University of Glamorgan's role was to demonstrate steady and non-steady state operation of laboratory-scale anaerobic and aerobic reactors and to develop a neural network control system. On-line monitoring and data acquisition, which are not discussed in detail here, were undertaken by a colleague in UG.

1.2 Dyes and Dyeing.

Dyestuffs are the materials from which colour, or dye, is produced. The majority of natural dyestuffs have poor fastness to washing and light. Therefore as textile and weaving technology advanced there became a demand for dyes that would give precise shades and were resistant to sunlight and mechanical and chemical processing (Grierson, 1989). The first synthetic dye was discovered in 1856 by W.H. Perkin, who obtained a purple precipitate called mauvene when trying to synthesise quinine from coal tar. Since that time over 7000 dyes and pigments have been synthesised (Aspin, 1981). By the end of the 19th century the use of synthetic dyes had been firmly established (Grierson,

1989). A history of dyes and important events in the textile industry from 2600 BC to the 20th Century can be found on the internet (Druding, 1998).

Modern textile dyes are required to have a high degree of chemical and photolytic stability (Easton, 1995). The stability refers to the ability of the dye to maintain its structure and colour and to resist breakdown due to time and exposure to sunlight, water, soap (McCurdy *et al.*, 1992), washing, bleach and perspiration (Travis, 1993) among other factors. Modern dyes are required to have a reproducible colour for commercial reasons. BASF require different batches of the same dye to exhibit the same colour within 2% (John Easton, pers. comm.). Dyestuffs derive their colour from their chromogens. A chromogen is an unsaturated system that either is coloured or becomes coloured as a result of substitution by atoms or groups. Chromogens are sometimes referred to as chromophores (Rattee, 1995).

Dyeing is the process by which dye is transferred from the dyebath to the fibre. Dyes and fibres to which they are applied usually have an attraction for each other which increases transfer of the dye from the dyebath to the fibre and often helps to fix the dye. The attraction of the dye to the fibre is referred to as the affinity of the dye for the fibre, although the dye also has affinity for the dyebath liquor. When a textile is immersed in a solution of dye, the dye is adsorbed to the cloth until a steady state has been achieved. The depth of the final colour of the cloth is determined mainly by the quantity of dye added to the fibre (Gutta, 1997). The percentage of dye in the dyebath that is adsorbed is referred to as the percentage exhaustion (Rattee, 1995). Good exhaustion is desirable for both economic and environmental reasons. The time required to achieve steady state is often longer than is commercially realistic, however, and therefore the exhaustion of the dye is less than it would be at equilibrium. Even at equilibrium, dye is never completely exhausted from the dyebath (Furr, 1992; Gutta, 1997). Higher temperatures enhance the diffusion of dye into fibres such as cotton by causing swelling of the cloth and generation of smaller dye aggregates than achieved at low temperatures (Gutta, 1997).

1.2.1 Dye Classes.

One well-known system of classification used internationally for dyes is the Colour Index (C.I.), devised by the Society of Dyers and Colourists in 1924. This classifies dyes by firstly assigning each a generic name determined by its application characteristics, and then assigning a C.I. constitution number based on its chemical structure, if known. Laing (1991) cites approximately 3000 dyes as being in use with 7000 formulations, although only about thirty of these are used at weights in excess of 1000 tonnes per annum. Ninety per cent of products listed are used at quantities of 100 tonnes per annum or less (Easton, 1995) and 80% of textile dye users are thought to use 200 kg or less of dye per annum (Laing, 1991). Therefore small amounts of a wide range of dyes are typically used together with large quantities of a small number of products. Dyes are selected according to the material to be dyed. The variety and chemical types of dyes used determine the nature of the effluent produced in textile manufacture. Table 1.1 illustrates the range of chemical types associated with different dye classes, the range of substrates with which each class may be used and their method of application. A description of the different dye types can be found in literature (e.g. Shore, 1990a; Diaper *et al.*, 1996).

Azo dyes are coloured chemical compounds containing one or more azo chromogens (-N=N-), the most common chromogen of textile dyes. Disazo and trisazo dyes have higher substantivity for cellulose (Shore, 1990a). Dyes from almost all application classes are included in the azo group (Table 1.1). Many azo dyes have one or more sulphonic acid groups as this group solubilises the dyestuff effectively (Stead, 1990a). If colorant precursors and sulphur dyes, which have indeterminate structures, are excluded then nearly two thirds of the organic colorants listed in the C.I. are azo dyes, one sixth of which are metal complexes (Easton, 1995). Azo dyes have been estimated to comprise 50 to 70% of all textile dyestuffs produced (Moll, 1991; Michelsen *et al.*, 1993; Carliell *et al.*, 1995; Geisberger, 1997; Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD), 1998). The importance of this chemical type in textile dyeing is therefore apparent.

Table 1.1 Dye Classes, Their Chemical Type, Range Of Substrates And Method Of Application (After Kirk-Othmer, 1993).

Class	Substrates	Method of Application	Chemical types
Acid	Nylon, wool, silk, paper, inks, and leather	Usually from neutral to acidic dyebaths	Azo including premetallised anthraquinone, triphenyl-methane, azine, xanthene, nitro, and nitroso
Azoic components and compositions	Cotton, rayon, +cellulose acetate, and +polyester	Fibre impregnated with coupling component and treated with a solution of stabilised diazonium salt	Azo
Basic	xPolyacrylonitrile-modified nylon, polyester, and inks	Applied from acidic dyebaths	Diazacarbocyanine, cyanine, hemicyanine, diazahemicyanine, diphenylmethane, triarylmethane, azo, azine, xanthene, acridine, oxazine, and anthraquinone
Direct	Cotton, *rayon, paper, leather, and nylon	Applied from neutral or slightly alkaline baths containing additional electrolyte	Azo, phthalocyanine, stilbene, and oxazine
Disperse	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions often applied by high temperature-pressure or lower temperature carrier methods; dye may be padded on cloth and baked on or thermofixed	Azo, anthraquinone, styryl, nitro, and benzodifuranone
Fluorescent brighteners	Soaps and detergents, all fibres, oils, paints, and plastics	From solution, dispersion, or suspension in a mass	Stilbene, pyrazoles, coumarin, and naphthalimides
Food, drug and cosmetic	Foods, drugs, and cosmetics		Azo, anthraquinone, carotenoid, and triarylmethane
Mordant	Wool, leather, and anodized aluminium	Applied in conjunction with chelating Cr salts	Azo and anthraquinone
Natural	Food	Applied as mordant, vat, solvent, or direct and acid dyes	Anthraquinone, flavonols, flavones, indigoids, chroman
Oxidation bases	Hair, fur, and cotton	Aromatic amines and phenols oxidized on the substrate	Aniline black and indeterminate structures
Pigments	Paints, inks, plastics, and textiles	Printing on the fibre with resin binder or dispersion in the mass	Azo, basic, phthalocyanine, quinacridone, and indigoid
Reactive	Cotton, wool, silk, and nylon	Reactive site on dye reacts with functional group on fibre to bind dye covalently under influence of heat and pH (alkaline)	Azo, anthraquinone, phthalocyanine, formazan, oxazine, and basic
Solvent	Plastics, gasoline, varnish, lacquer, stains, inks, fats, oils, and waxes	Dissolution in the substrate	Azo, triphenylmethane, anthra-quinone, and phthalocyanine
Sulphur	Cotton and *rayon	Aromatic substrate vatted with sodium sulphide and reoxidised to insoluble sulphur-containing products on fibre	Indeterminate structures
Vat	Cotton, *rayon, and wool	Water-insoluble dyes solubilized by reducing with sodium hydrosulphite, then exhausted on fibre and reoxidised	Anthraquinone (inc. polycyclic quinones) and indigoids

*Rayon now referred to as viscose

+Azoics no longer used on polyester and cellulose acetate

xShould read basic-dyeable nylon

1.2.1.1 Reactive Dyes and Dyeing.

Reactive dyes are water-soluble anionic dyes containing at least one chemical group, the reactive group, that is able to form a covalent bond with the fibre (Diaper *et al.,* 1996). The first commercial reactive dyes were launched by ICI in 1956 under the name PROCION. They exhibit good wash fastness as the energy required to break the covalent bonds is in the range of 70-200 kJ mol^{-1} (Lewis, 1998). Reactive dyes can be based on a variety of structures (Table 1.1) with azo dyes accounting for over 95% of reactive dyes, with the exception of other structures in the bright blue-green colours. Reactive dyes are usually unmetallised but some metal complexes exist in darker shades such as navy, brown and black (Shore, 1990a). Reactive dyes are principally used with cellulose although the dyes can also react with other fibres such as nylon, wool and silk (Table 1.1). Reactive dyes have one or more leaving groups, located on the reactive group, the most common of which is chlorine. Other leaving groups include quaternary ammonium and sulphone groups, and fluorine (Stead, 1990b).

Reactive dyes are estimated to account for 10-11% of dye used world-wide but in more developed countries the percentage of reactive dyes used is greater (Upton and Churchley, 1997). They were cited as being used to dye approximately 25% of all cotton in 1990 (Stead, 1990b) and that percentage had increased to 32% in 1993 (Holme, 1997). Gordon (1998) cited a world-wide reactive dye consumption of 109,000 tons per annum in 1992 compared to 60,000 tons p.a. in 1988, not including China, India and Eastern Europe. Growth rates for reactive dye usage are predicted to be greater than those for cotton fibre (Section 1.1) at about 4% p.a. (Reddig, 1997) with a consumption of 178,000 tons p.a. anticipated for 2004 (Gordon, 1998). This is due to such factors as their wide range of colours and good wash fastness, and technical and economic limitations associated with other dyes which has resulted in substitution of reactive dyes for other dye classes. Due to the increase in reactive dye consumption pollution associated with these dyes will also increase.

The dyeing of cotton with reactive dyes has a number of stages. First the dye is exhausted onto the cloth in the presence of an electrolyte such as sodium chloride at neutral pH. Under these conditions physical adsorption and some hydrogen bonding occur (Trotman, 1989), which result in the dye molecules being ideally sited for reaction with the fibre. The recommended electrolyte concentration varies with the dyestuff used, its substantivity for the fibre, depth of shade and liquor ratio (Sewekow, 1993), and increases with temperature. Some cited concentrations of common salt in the dyebath range from 5 to 140 g l^{-1} (Goronszy and Tomas, 1992; Sewekow, 1993; Lockerbie, 1994; Thakur *et al.* 1994). Standard dyeing conditions for non-mercerised cotton are cited as a solution of 2% dye and 50 g l^{-1} salt with a liquor ratio (kg dyebath:kg fibre) of 10:1, at a temperature of 60°C (International Dyer, 1998). Mean reported concentrations of dye added to reactive dyebaths vary from 20-80 g l^{-1} (Thakur *et al.* 1994) to 50-100 g l^{-1} (Perkins, 1996) with the concentration determined by the final colour required.

After adsorption of the dye has occurred alkali is added, leading to ionisation of hydroxy groups on the cellulose molecule (Stead, 1990b). Nucleophilic cellulosate anions are thus formed which can react with the dye (Lewis, 1998) to form covalent bonds. Nucleophiles are reagents with electron-rich sites that form a bond by donating electrons to an electron-poor reagent. Hydroxide ions may also act as attacking nucleophiles resulting in hydrolysis of the dye (Stead, 1990b). This results in the effective wastage of some of the dye as it is then unable to react with the fibre (Trotman, 1989). Fixation predominates over hydrolysis, however, because the dye molecules have already been adsorbed onto the fibres and are therefore ideally sited for reaction with them (Stead, 1990b). The rate of reaction of reactive dye with water has been found to be slower than that with a range of organic compounds. Therefore the dye should generally have time to react with the cellulose before excessive hydrolysis occurs (Trotman, 1989). The rate of hydrolysis increases as the temperature rises, and hence the substantivity ratio (the relative concentrations of dye absorbed onto the substrate and remaining in the dyebath) decreases (Shamey and Nobbs, 1997). This must be off-set against the greater swelling of the cloth and better diffusion of dyes at higher temperatures (Section 1.2). Once the dye has reacted with the fibre the cloth is washed to remove electrolyte, alkali and unfixed dye (Shamey and Nobbs, 1997). Steenken-Richter and Kermer (1992) reported that up to

0.8 g l^{-1} of hydrolysed dye could remain in the bath after completion of the reactive dyeing process while Gähr *et al.* (1994) gave 0.1-0.2 g l^{-1} as the normal range of reactive dyebath residues.

1.2.2 Dye Fixation.

Dye losses from textile processing operations are determined by a number of factors including the dye type, shade depth, method of application, and liquor ratio (Laing, 1991). The degree of fixation for different dye and fibre combinations can be seen in Table 1.2.

Table 1.2 Estimated Degree Of Fixation For Different Dye-Fibre Combinations (After Easton, 1995). Also The Loss To Effluent Given By a) Easton (1995) And b) Laing (1991).

Dye Application Class	Fibre	Degree of fixation (%)	a) Loss to effluent (%)	*b) Loss to effluent (%)
Acid	Polyamide	80-95	5-20	7-20
Basic	Acrylic	95-100	0-5	2-3
Direct	Cellulose	70-95	5-30	5-20
Disperse	Polyester	90-100	0-10	1-20
Metal-complex	Wool	90-98	2-10	2-5
Reactive	Cellulose	50-90	10-50	20-50
Sulphur	Cellulose	60-90	10-40	30-40
Vat	Cellulose	80-95	5-20	5-20

*The fibre type used with each dye was not specified in b.

The highest fixation rates are achieved with basic dyes. It is seen that reactive dyes exhibit rather low rates of fixation, with up to 50% being discharged in the effluent. Effluent from cotton processing therefore compares poorly in terms of colour to, for example, effluent from the wool industry where up to 98% of the dye applied to the material is absorbed (Table 1.2), resulting in effluent that is far less coloured. Fixation rates for reactive dyes tend to be higher in dyes containing two or more reactive groups (Kirk-Othmer, 1993; Carr, 1995). Dye manufacturers are attempting to increase the rate of fixation of reactive dyes by reducing the quantity of dye hydrolysed (Stead, 1990b).

The popularity of different dye types in the textile industry can be seen by examining the percentage of each class used in two catchments in the UK (Table 1.3). When this information is taken into account together with the dye losses to effluent an estimate is obtained of the impact of each dye class on water pollution in the UK.

Table 1.3 Percentage Use Of Dyes By Class In Two Catchments In The Severn Trent Water Treatment Catchment (Adapted From Churchley, 1994).

Dye Class	Loughborough Catchment (%)	Wanlip Catchment (%)
Reactive	60.8	66.7
Metal	2	4
Disperse	14.9	7.2
Basic	1	6
Acid	5	4.8
Sulphur	4.2	9
Direct	11.4	1.5
Mordant	1	0

The dye types used varied with catchment due to the variety of textiles processed in each area (Table 1.3). However, reactive dyes were dominant in both catchments (O'Neill *et al.*, 1999a). It can be seen that reactive dyes present the greatest problem in terms of colour in effluent due both to their popularity and poor fixation rates. Thus Table 1.3 emphasises the importance of treatment of effluent from cotton processing. The manufacture of cotton textiles is examined in the following section to illustrate the different stages of production, some of which give rise to effluent.

1.3 Production of Cotton Textiles.

Effluent can be generated from many of the processes involved in cotton textile production. Cotton is delivered to the factories in bales. The fibres are then sorted depending on their grade, cleaned of impurities such as soil and leaves, and blended to improve the consistency of the fibre mix. These processes are referred to as opening and blending. This is followed by carding which removes shorter fibres that would weaken the yarn and aligns the fibres into thin parallel sheets to prepare them for spinning. The

product is referred to as carded sliver and is combed to produce finer and cleaner comb sliver. Several slivers are combined and fed to a drawing frame which draws and lengthens them, extending them to five or six times their original length. Slivers from different types of fibres, e.g. cotton and polyester, may be combined during this process to form fibre blends. Drawing is followed by drafting which further stretches the yarn and gives it a slight twist. The yarn is now referred to as roving. Rovings may be blended with other fibres if required, and are then drawn further and spun. The spun fibre can be woven, knitted or tufted into fabric (Environmental Protection Agency, 1996). Prior to weaving, the warp yarn must be sized with macro-molecular substances in order to protect it from the stresses of weaving (Grau, 1991; Smith, 1996). Weaving is the most common method of production of cotton fabrics and modified starch is usually used as the sizing agent. The woven fabric is known as grey or greige fabric. The procedures referred to as 'finishing' are carried out on the greige. Finishing generally refers to desizing, dyeing, printing and applying finishes to the fabric (Watson, 1991).

Treatments for desizing, washing and scouring the cloth are carried out on the greige. Desizing removes the size from the woven cloth to permit better and more even dyeing of the material. It can be carried out with sodium hydroxide or enzymes (Cardamone and Marmer, 1995). Scouring removes substances such as natural waxes and pectins, herbicides and pesticides used in the growth of cotton, and spinning oils. It is usually performed with hot alkaline solutions such as caustic soda or soda ash, together with detergents or soaps. Following scouring the cloth is bleached either to produce white cotton or prepare the material for dyeing. Bleaching is normally carried out using sodium hypochlorite, sodium chlorite, or hydrogen peroxide (Milner, 1997). Peroxide is used for 90% of bleaching in the UK (Smith, 1996).

Almost all dyeing and printing processes occur by the application of a solution or dispersion of the dye to the material followed by fixation (Shore, 1990b). Dyeing with reactive dyes is described in Section 1.2.1.1. Dyeing is followed by two baths which bring unfixed dyes to the fibre surface so that they then can be washed away. The first removes the alkali and salt, while the second acidifies the material to prevent fixed dyes from becoming partially hydrolysed during subsequent stages (International Dyer, 1998).

The product may then be subjected to a range of procedures including bulking and texturising, optical finishing, brushing and napping, softening, shearing and compacting (Environmental Protection Agency, 1996). It can be seen from the wide range of processes involved in textile production that many chemicals are used in manufacture of cotton fabric and may be present in the final effluent.

1.4 Textile Effluent.

Textile effluent varies from day to day and even hour to hour, due to the batchwise nature of the dyeing process, and is therefore difficult to characterise. Large quantities of effluent are generated with varying composition depending on the wet processes (Section 1.3). The final effluent varies with fibre type, chemicals used, season, and customers' orders. Therefore fashion has a role to play in determining the nature of textile effluent as it affects the fabrics and colours used and hence the dye types (O'Neill *et al.,* 1999a). In many factories wastewater flow is intermittent and variable and may only occur five days a week (Gardiner and Borne, 1978). There are three main divisions of water utilisation in the textile industry: steam (5-10%), cooling water (25-35%), and industrial water (50-60%) (Durig, 1981).

Textile wastewater is generally relatively hot with a final effluent temperature of 35-40°C (Lin and Lin, 1993). It tends to contain non-biodegradable dyes and toxic substances including metals (Correia *et al.,* 1994) in addition to high concentrations of inorganic and organic chemicals (Altinbas *et al.,* 1995). The large number of compounds contained in textile effluent was demonstrated by Alaimo *et al.* (1990) who positively identified 314 compounds, determined the partial structure of 94, and detected an additional 107 unknown compounds in wastewater streams from four factories. The effluent is typically grey (British Textile Technology Group, 1996) although the colour depends on the mixture of dyes being used in the factory at the time. Cotton processing effluents are highly coloured and have high concentrations of total dissolved solids, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Correia *et al.,* 1994).

Abo-Elela *et al.* (1988) found a mean pH of 8.3, COD of 974 mg l^{-1} and BOD of 396 mg l^{-1} in end-of-pipe effluent from a textile mill carrying out fabric finishing, although there was substantial variation in these parameters. Meyer *et al.* (1992) found dyeing and finishing effluent to have a COD of 1300 mg l^{-1} and a pH of 7.3. Another review found mixed dyehouse wastes to have a BOD of 200-3000 mg l^{-1}, COD of 500-5000 mg l^{-1}, suspended solids of 50-500 mg l^{-1} and pH of 4-12 (Laing, 1991). Correia *et al.* (1994) cited BODs of 0 mg l^{-1} for batch reactive dyeing wastewater and 120 mg l^{-1} for continuous reactive dyeing effluent, with pHs of 11.2 and 9.1 respectively. The average BOD and TOC of dyehouse effluent have also been cited as 280 and 276 mg l^{-1} respectively (Laing, 1991). In the carpet industry dyebath chemicals comprised 25-35% of the COD, with dyes forming 2-5%. It can be seen that the organic load in textile effluents varies greatly. An American study found that dyeing wastes generally comprised 10-30% of the BOD of textile mill waste water and 1.5-30% of the total BOD in the cotton industry (Laing, 1991). The dyeing process has been estimated to comprise 5% of the BOD loading (Park and Shore, 1984). Most dyes exhibit low BOD and low toxicity. They thus tend to be less polluting than substances such as sizing agents, waxes and impurities from raw cotton, despite the colour they impart to effluent (Durig, 1981).

In Egypt the National Textile Company found desizing and scouring to be the most polluting processes in a plant processing spun yarns (Hamza and Hamoda, 1980). In addition to size, desizing effluent may contain additives used in the size recipes, surfactants, enzymes, acids or alkalis (Correia *et al.*, 1994). Desizing effluent from the cotton industry generally comprises about 16% of the total wastewater volume (Etzel, 1985) and has a high pollutant load, giving rise to high COD in cotton processing effluent. It has been cited variously as containing between 1.5-75% of the effluent BOD (Park and Shore, 1984; Etzel, 1985; Stegmaier *et al.*, 1998) and 50-55% of the COD (Smith, 1996; Weber and Ströhle, 1997). However, it has been estimated to account for only 6% of the water consumption (Park and Shore, 1984). The enzymatic removal of starch generates approximately 60 kg BOD per 1000 kg of fabric (British Textile Technology Group, 1996). Where acetic acid is used as an acidifying agent it can account for 50-90% of dyehouse BOD (Laing, 1991). Additional sources of COD include fibre decomposition products, other chemicals used and by-products of the dyeing and finishing processes

(Milner, 1997). It has been suggested that ecological problems caused by textile effluents might be reduced if the sizing agents or blends had a biodegradability of 80% or greater (Coia-Ahlman and Groff, 1990). However, large quantities of easily degradable substances, such as starch sizes and organic acids, can also have significant environmental impact.

Dyebath additives such as salt may have an environmental impact and therefore affect the discharge consent conditions and effluent treatment costs (Laing, 1991). There is pressure on dye manufacturers to develop dyes that can be successfully applied using less auxiliary chemicals, particularly salt, to reduce environmental problems associated with textile industry effluent (Carr, 1995). Typical concentrations of common salt in dye effluents have been reported to range from 2-3 g l^{-1} (Ghorpade and Spencer, 1993; Rucker and Guthrie, 1997) to 5 g l^{-1} salt in reactive dyeing effluent (Sewekow, 1993). Some industrial effluents contain 6.6-11.5 g l^{-1} (P. Flannery, pers. comm., Fruit of the Loom, Co. Donegal, Ireland) and 8 g l^{-1} NaCl (Levi Strauss (Belgium) - J. Liessens, Biotim, Belgium, pers. comm.). Textile effluents, including those from cotton textile manufacture, may also contain substances such as solvents, grease, machine cleaning products, biocides and insecticides, some of which may be difficult to biodegrade (Cooper, 1992). Biocides can kill the bacteria necessary for biological degradation and hence wastes containing such substances are not suitable for biological treatment. The composition of textile effluent in terms of potentially toxic substances therefore has to be known prior to determining a suitable method of treatment for such effluents.

1.4.1 Colour in Textile Effluents.

Coloured effluents have probably been produced since the first dyeing operations began. However, initial operations were on a very small scale and therefore were likely to have had only localised effects on the environment. Today textiles are produced on a large scale and hence the large quantities of coloured effluent generated have global impact. Problems associated with colour in textile effluent have been of concern both industrially and academically for at least four decades in most industrialised countries (Southern,

1995). The variation of colour in textile effluent gives rise to fluctuation of the COD and pH as both parameters vary with the types and quantities of dye present in the effluent (Lin and Lin, 1993; Lin and Peng, 1994). The combination of colour and high concentrations of dissolved solids results in high turbidity of the effluent (Lin and Lin, 1993).

Over the whole range of application classes approximately 10% of dyes used for deep shades are lost, 2% of medium shades and very little from pale shades (Laing, 1991). In 1978 it was estimated that 2% of the 450,000 tonnes of dye produced world-wide were discharged in effluent from dye manufacturing operations. Approximately 9% was discharged in effluents from the coloration industries, giving a final total of almost 50,000 tonnes (Brown, 1987). Dye consumption has increased since 1978 but dyes and dyeing processes have been continually improved to try and reduce the quantities lost to effluent. A more recent report cited ~12% loss of synthetic textile dyes during manufacturing and processing, 20% of which entered the environment, i.e. 2.4% (Weber and Stickney, 1993). Currently ~20 million tonnes of cotton are produced annually and dyed with an average 2% depth of shade giving a total dye consumption of ~400,000 tonnes (Smith, 1996). If it is assumed that reactive dyes are used to dye 32% of all cotton as in 1993 (Section 1.2.1.1) then this corresponds to 128,000 tonnes reactive dye. A dye wastage of 10-50% gives rise to an annual discharge of 12,800-64,000 tonnes in effluent from cotton processing. Obviously, the higher the fixation rate the lower the quantities of dye discharged. These figures give some impression of the scale of the problem of colour from reactive dye in effluent.

There are few reports of measured dye concentrations in rivers. However, the predicted environmental concentration of a dye in receiving waters from a dyehouse can be calculated from the daily dye usage, degree of fixation on the substrate, degree of removal during treatment processes, and the dilution by the receiving water (Easton, 1995). Laing (1991) reported typical dye concentrations of 0.01-0.05 g l^{-1} in dyehouse effluent. Several other authors reported that a dyehouse dyeing cotton with reactive dyes would be expected to discharge 0.06 g l^{-1} of dyestuff (Glover *et al.*, 1992; Sewekow, 1993; Pierce, 1994; Shelley 1994). This is cited as being obtained at a colour depth of 2% and a water

consumption of 100 l kg^{-1} goods dyed and rinsed (Sewekow, 1993). Other figures cited are 0.06 g l^{-1} when reactive dye is applied by exhaustion in a jet dyeing machine, and ~0.25 mg l^{-1} when applied continuously using a pad mangle (Glover *et al.*, 1992). Vandevivere *et al.* (1998) cited 0.6-0.8 g l^{-1} dye as being present in dyehouse effluents. Kace and Linford (1975) cited a typical dye concentration of 0.1 g l^{-1} in textile effluent. Therefore it can be seen that there is great variation.

Ghorpade and Spencer (1993) gave typical intensities of colour in dye wastes as 1000-1500 American Dye Manufacturers Institute (ADMI) units. Goronszy and Tomas (1992) noted that effluent from a Puerto Rican plant dyeing cotton and 50% polyester/cotton had a colour of 38 to 2000 ADMI units over a 12 week period, with a mean of 797 units. Correia *et al.* (1994) cited ADMI values ranging from below 50, for effluent from dyeing of nylon with a range of dye types, to 12500 units for effluent from direct dyeing of viscose. ADMIs of 1390 and 3890 units were cited for reactive dye wastewater from continuous and batch processes respectively.

1.4.2 Colour Discharge Consents.

The impact of dye pollution is assessed from the quantities discharged, their toxicity to fish and micro-organisms, and their accumulation, both in nature and in the food chain (Meyer, 1981). However, it has been found that textile dyes present in normal concentrations in wastewater are not usually toxic or inhibitory to aquatic flora and fauna, or to biological treatment plants (Durig, 1981; Churchley, 1998). Clarke and Anliker (1984) surveyed ~3,000 colorants and found only 2% had an LC_{50} to fish of <1.0 mg l^{-1}. The vast majority had LC_{50} values in excess of 100 mg l^{-1}. Additionally, it has been reported that there is little evidence for bioaccumulation of the more commonly used dyes (Diaper *et al.*, 1996). Hence it was concluded that most dyes are not toxic. However, the colour that they impart to water gives rise to complaints (Durig, 1981). The eye can detect concentrations of 0.005 mg l^{-1} dye (Pierce, 1994; Shelley, 1994) and therefore concentrations exceeding this would not be permitted on aesthetic grounds. Hence

consent levels for the discharge of colour to receiving waters are normally applied for aesthetic reasons and not for prevention of toxicity.

Rivers in the UK Midlands have improved in quality since the 1950s due to improvements in water treatment. This has resulted in decreased turbidity of river water and hence increased visibility of coloured pollutants. Therefore since the mid 1970s there have been complaints about colour in some rivers in the UK Midlands (Upton and Churchley, 1997). The fashion for brilliant hues in full depths on cotton has resulted in an increase in the quantities of reactive dyes used. This in turn has contributed to the increased number of complaints from the public regarding coloured water discharged to rivers downstream of sewage works (Churchley, 1994). In 1992 colour was the subject of more than 500 complaints in the UK, mostly from the Severn Trent region (Pierce, 1994), and up to 1995 accounted for about 5% of complaints received by the National Rivers Authority (NRA) of the UK, now part of the Environment Agency (EA) (Waters, 1995). Colour in textile effluents, particularly red hues, is usually linked to the presence of reactive azo dyes in the water (Carliell *et al.*, 1995) and reflects the large quantities of such dyes that may be discharged (Section 1.4.1). Most complaints concerning colour tend to refer to water containing red dyes (Smith, 1996). There are far fewer complaints regarding colours such as blue, green or brown as these are colours that might be expected of rivers or are less noticeable (Easton 1995). Due to such complaints, new regulations were introduced to place pressure on Water Companies to reduce colour in sewage effluent. In 1989 the NRA announced their intention to tighten the consent conditions for Sewage Treatment Works (STW) in the Severn Trent catchment area, including those for colour (Upton and Churchley, 1997).

Consent conditions are agreed between the regulating body and the producer of the effluent and those for colour are determined by taking into account the dilution in dry weather conditions and the average upstream colour. It is not feasible to express colour in mg l^{-1}, since similar concentrations of different dyes can produce totally different results in terms of both colour and intensity (Waters, 1995). Therefore absorbance and, less commonly in this country, ADMI values are used to express colour limitations on discharge. Another method becoming more popular in Europe is measurement of the

spectral absorption coefficient ((absorbance/pathlength)x1000) at three specified wavelengths according to ISO 7887, 1994 (British Standards Institution, 1995). Another version of this is the true colour measurement, the mean absorbance at the three specified wavelengths. The usual practice in the UK for determination of consents for a river to which effluent is to be discharged is to take samples of river water and assess which are acceptable to the eye. The absorbance of these samples in a 1 cm cell between 400 and 700 nm when filtered through a 0.45 μm filter is then measured and the results used as a basis for the determination of consent limits. Consent limits are usually expressed as absolute limits at 50 nm intervals (Waters, 1995). Exceptions may be made if significant intermediate peaks are present but generally the presence of narrow peaks is unlikely as factories tend to produce effluent containing a wide range of dyes (Hazel, 1995). The colour standard for the river must be met at times of low flow conditions upstream when the river upstream is of normal colour and average daily effluent flows are being discharged to the river. Some colour consent values for STWs receiving coloured trade effluents can be seen in Table 1.4.

Table 1.4 Colour Consent Standards For STW Receiving Coloured Trade Effluents In Three Sewage Works In The Severn Trent Catchment Area (After Upton And Churchley, 1997) And 'Typical' Consent Values (EA).

Wavelength (nm)	Leek (abs)	Wanlip (abs)	Pinxton (abs)	Typical colour consent* (abs)
400	0.060	-	-	0.115
450	0.040	-	-	0.085
500	0.035	0.020	0.028	0.065
550	0.025	0.021	0.025	0.055
600	0.025	0.012	0.024	0.040
650	0.015	0.012	0.017	0.028
700	-	-	-	0.013

*supplied by the Environment Agency (Shrewsbury, UK).

It was seen that the colour consents for Leek, Wanlip and Pinxton were all lower than the typical colour consent provided by the EA. This was due to the presence of many textile works in the Severn Trent area, which send effluent to be treated in STWs. The quantity of colour each sewage works could discharge to the rivers was thus limited (O'Neill *et al.*,

1999a). The 'typical' colour consent came from a region where there were few dyeworks in the locality and therefore was higher than consents for the other works. Hence regions with several dye works in the area and/or small rivers may find it difficult to meet the consents set by the authorities (Pierce, 1994).

1.5 Treatment.

In the UK 80-90% of textile finishers send their effluent to be treated in local sewage treatment works (Hazel, 1995; Smith, 1996; Moran *et al.*, 1997). The remainder treat their own waste. When discharged to sewer, textile effluents are treated with domestic and other industrial effluents and released to surface waters (Easton, 1995). Domestic sewage buffers the pH of industrial effluent, dilutes inhibitory materials, and provides nutrients such as nitrogen and phosphorus, which may be too low in the trade effluent to allow adequate bacterial activity (Churchley, 1995). Therefore it can enhance degradation of such effluents. One study recommended that textile effluent should be mixed with at least 50% domestic sewage to compensate for nutrient deficiency in the former and hence produce a good quality final effluent by means of activated sludge treatment (Abo-Elela *et al.*, 1988). Additionally, Van Baardwijk *et al.* found 50% of the chemicals in dyeing and finishing wastewaters to be unaffected by biological treatment (Coia-Ahlman and Groff, 1990). In 1990 Severn Trent Water reported that sewage works were ineffective at removing reactive dyes, among other substances, and passed the responsibility for waste treatment onto the individual industries (Harrison, 1995). This trend is likely to increase as legislation becomes more strict.

The strong colour of textile wastes is the hardest component of the effluent to treat (Lin and Lin, 1993; Cowey, 1998). For example, recently a fashion for black-dyed jeans resulted in the production of a problematic highly coloured effluent in a Belgian factory (O'Neill *et al.*, 1999a). Hence the treatment of dyeing effluent is considered to be one of the most difficult wastewater treatment technologies (Park and Lee, 1996). Colour removal is therefore said to be one of the most difficult problems for environmental engineers in the design of treatment facilities (Yeh and Thomas, 1995a). The difficulty is

attributable to the colour fastness, stability and resistance of dyes to degradation (Section 1.2). Therefore even after treatment in a STW, the effluent may still be coloured, which may lead to complaints (Section 1.4.2). Although the dyes are unlikely to adversely affect the environment to which they are discharged (Section 1.4.2), they may be of concern when the treated effluent is used as a supply of drinking water (Loyd *et al.*, 1992). Removal of over 99% of colour, despite varying strength and volume, may be required (Hoyle, 1995). However, dilution of textile effluent to the point where colour is no longer apparent would require huge amounts of water. Therefore treatment of colour is essential. The pressure being put on textile manufacturers to treat their effluent has led to the development of a range of technologies competing to provide cost-effective solutions (Laszlo, 1994). The use of simulated textile effluents can be advantageous in the investigation of treatment technologies as they enable research to be carried out in the absence of a local source of effluent. They also have constant composition and therefore facilitate the assessment of treatment methods without interference from the fluctuations associated with real textile effluents (O'Neill *et al.*, 1999a).

Physical, chemical and biological treatment methods can be used singly or in combination, the effluent composition determining the most effective treatment. Removal of dyes and their intermediates can often be achieved only through a combination of treatments (McCurdy *et al.*, 1992). It has been reported that textile effluents are difficult to treat either biologically or by combinations of biological, chemical and physical methods (Lin and Lin, 1993). The large variation in pH of textile effluent (Laing, 1991; Section 1.4) adds to the difficulty as the pH tolerance of many treatments is limited (Lin and Chen, 1997). Due to the potential health risks associated with some of the chemicals or additives contained in textile effluent, any sludge formed could be hazardous and should be disposed of carefully (Southern, 1995). This adds to the cost of treatment.

The principal concerns of the textile industry regarding treatment are the speed of decolourisation; ease with which it is achieved; percentage colour removal; cost; potential for reutilisation of the effluent; and the environmental impact of the techniques (Uygur, 1997). It has been suggested that effluent from different processes be treated separately in order to achieve maximum treatment efficiency (Boudreau, Dubeau, Lemieux Inc.,

1981) for parameters such as colour (Gubser, 1972). However, usually effluent from different processes is mixed. Many companies find equalisation and holding of wastewater reduce variation in water quantity and quality and thus increase the treatment capacity of plants (Altinbas *et al.*, 1995; R. Bianchi, CIDA, Italy, pers. comm.). It was thought that it might be possible to coagulate textile effluent and feed the coagulated material to an anaerobic digester. This would result in small quantities of a highly concentrated effluent entering the anaerobic system. Therefore the physical-chemical treatments of coagulation and flocculation are examined here along with the treatment of textile effluents by biological means, comprising anaerobic treatment, aerobic treatment and combined treatment methods.

1.5.1 Coagulation and Flocculation.

Coagulation and flocculation can be used to achieve colour removal from textile effluents. They can be carried out alone or in conjunction with other treatments. Reactive dyes may be removed by means of coagulation/flocculation techniques (Willmott *et al.*, 1998). Courtaulds Textiles (UK) found that such treatment of raw balanced effluent could remove colour to a degree that complied with the standard consent for discharge to sewer (Cooper, 1993). In addition to such tests on real effluents, a number of authors have performed studies on use of these techniques with simulated wastes including Kace and Linford (1975), Koprivanac *et al.* (1992, 1993) and Kang and Chang (1997).

Coagulation is the destabilisation of colloids in solution (Degrémont, 1991). Colloids are particles which are too small to settle on standing. Coagulation enables the particles to approach each other resulting in their agglomeration into larger, finely dispersed stable particles (Arvin and Henze, 1995) which may grow to 2 mm in diameter (Hoyle, 1995). Adequate mixing is required to ensure that there is sufficient contact between the coagulant and the solution (Hall, 1997). Settling often does not occur with the use of coagulants alone (Arvin and Henze, 1995). Therefore flocculation may also be required.

Flocculation is the agglomeration of the flocs formed by coagulation into larger clusters by the addition of flocculants which cause them to stick together upon collision (Arvin and Henze, 1995). The flocs can be settled, floated, or filtered to achieve separation. Agitation of the solution is required to cause collision of the particles but can cause the flocs to fragment if excessively vigorous. Temperatures over 20°C have a negative effect on the flocculation process and therefore warm effluents may need to be cooled. Flocculation is also strongly pH dependent, the optimum pH depending on the physical and chemical nature of the flocculant (Koprivanac *et al.,* 1992) and hence either the flocculant should be selected with regard to the pH of the effluent to be treated, or the pH of samples should be adjusted.

Inorganic chemicals, modern synthetic polymers and some organic and natural substances may be used as coagulants and flocculants (Degrémont, 1991). Coagulation by traditional methods uses large quantities of inorganic chemicals, results in the formation of large quantities of sludge and satisfactory results are not obtained with all dyes (Laing, 1991). The concentrations of synthetic compounds used are far less than those of traditional coagulants and give rise to a comparatively small quantity of sludge (Churchley, 1995). It has been reported that poor flocs are achieved if too little coagulant is added. However, addition of an excess results in production of more solids and, under some circumstances, weak flocs that are difficult to remove (Hall, 1997). Therefore coagulant dosage must be calculated carefully when possible. The variability of textile effluent composition may render this difficult. The advantages and disadvantages associated with coagulation and flocculation can be seen in Table 1.5.

Table 1.5 Advantages And Disadvantages Of Coagulation/Flocculation.

Advantages	Disadvantages
Easily retrofitted to existing treatment plants	Dilution of the textile effluent in STWs reduces the treatment efficiency
Does not usually require high capital input	High running costs
Do not generally adversely affect biodegradability and can thus be used either before or after biological treatment	Coagulants containing sulphate may lead to toxicity in anaerobic systems
Colour removal is rapid	Polyelectrolytes can be detrimental to activated sludge treatment
COD is decreased significantly	Large volumes of sludge may be generated depending on the chemicals used
	Not applicable for all waste types

1.5.2 Biological Treatment.

Most materials of plant or animal origin can be degraded by bacteria, fungi, or other organisms to simple compounds such as water, carbon dioxide and/or methane. Biological treatment processes use these natural reactions to degrade effluents and are usually the most economical means of removing the majority of pollutants from high-strength organic effluent (Razo-Flores *et al.*, 1996). There are two forms of biological treatment: anaerobic and aerobic. Anaerobic treatment is the conversion of waste by micro-organisms to methane and carbon dioxide in the absence of free oxygen. Aerobic treatment is the conversion of organic matter by micro-organisms to water and carbon dioxide in the presence of free oxygen. Municipal sewage treatment works are typically based on activated sludge treatment. In many cases anaerobic digestion is then used to reduce the quantity of aerobic sludge for disposal. Many anaerobic bacteria, but only a few aerobic bacteria, are capable of azo dye reduction (Chung *et al.*, 1993). Azo bonds are not usually made by living organisms and therefore knowledge about their biodegradation in nature is limited (Paszczynski *et al.*, 1991). The effect of different pollutants found in textile effluents on biological treatment can be seen in Delée *et al.* (1998).

1.5.2.1 Anaerobic Treatment.

Anaerobic treatment has been used since the beginning of the 20th century for the treatment of organic solids from domestic sewage (Holder *et al.*, 1975). It was originally used to treat high-solids, carbohydrate-rich wastes but it was thought the sensitivity of the methanogens would render this method inappropriate for the treatment of potentially toxic industrial wastes. In the last twenty years advances in reactor design and increased understanding of the process, among other things, have led to the more widespread use of anaerobic digestion, which is increasingly being used to treat industrial wastes (Terzis, 1994; Watson-Craik and Stams, 1995). Anaerobic treatment has had mixed success in the textile industry (Cowey, 1998) and is not yet well established in the treatment of textile effluents. However, some successful pilot scale and full scale plants have been reported (Delée *et al.*, 1998). Often anaerobic digestion needs to be part of a treatment series, rather than the sole method of treatment.

Anaerobic digestion is carried out by three major groups of micro-organisms: hydrolytic/ fermentative bacteria, obligate hydrogen-producing acetogenic bacteria, and methanogenic bacteria. The main stages in anaerobic decomposition can be seen in Speece (1996). They include hydrolysis of high molecular weight carbohydrates, fats and proteins into simple organic compounds by the enzymatic action of hydrolytic/fermentative bacteria followed by their fermentation to butyric, propionic and other long chain fatty acids. Some hydrogen gas (H_2), carbon dioxide (CO_2) and acetate are also formed. The fatty acids are then converted into acetic acid, H_2 and CO_2 by the hydrogen-producing acetogenic bacteria. Finally methanogenic bacteria convert acetic acid into CO_2 and methane (CH_4). Some CO_2 and H_2 are also converted to CH_4 by the methanogenic bacteria. Methanogens can grow on a limited range of carbon compounds, such as acetic acid, and are said to be the most sensitive micro-organisms participating in anaerobic treatment. Acetic acid is the principal volatile fatty acid (VFA) formed by anaerobic digestion (Rozzi *et al.*, 1997) and approximately 70% of methane production is attributable to its degradation (Jeris and McCarty, 1965). Methane typically comprises 75-80% of the gas produced (Olthof and Oleszkiewicz, 1982). The theoretical methane production is 0.35 l CH_4 g^{-1} COD removed at standard temperature and pressure (STP)

(Olthof and Oleszkiewicz, 1982). The quantity of COD converted to methane depends on factors such as the food:micro-organism ratio (F:M), temperature, the organisms present and hydraulic retention time (HRT) (Speece, 1996). Imbalances of the bacterial populations can lead to build-up of degradation products which can cause inhibition (Chynoweth *et al.*, 1994).

A constant temperature is required for efficient operation of anaerobic processes. Anaerobic treatment uses one of three types of bacteria, each with its own optimal temperature: psychrophilic (15°C), mesophilic (35°C) and thermophilic (55°C). At higher temperatures bacteria exhibit higher growth and organic removal rates (Olthof and Oleszkiewicz, 1982). An increase in VFAs may be found with a decrease in temperature as the rate of metabolism of the acetogens is less affected than that of the methanogens (Speece, 1996). Propionic acid is an important intermediate in the degradation of carbohydrates and proteins to acetic acid and then methane (Jeris and McCarty, 1965) and low concentrations of this acid indicate stability of the anaerobic system. High concentrations of acetate and hydrogen inhibit the conversion of propionic acid to those end products. Therefore high concentrations of propionic acid indicate inhibition within the system. Such inhibition leads to a build-up of VFAs, which leads to a decrease in pH if the buffering capacity of the system is exceeded. Inhibition also increases with decreasing pH. Other causes of high VFAs include trace metal limitation, toxicity, overload, and N or P limitation. The precise cause of high VFAs can be difficult to determine as the symptoms of toxicity and of trace metal deficiency are often relatively similar (Speece, 1996). Trace elements required include iron, cobalt and nickel, the latter of which appears to be the most stimulatory when added singly (Forster, 1991).

Bicarbonate alkalinity buffers anaerobic systems against changes in pH, such as those caused by increased VFA concentration. Suggested concentrations of bicarbonate range from a recommended minimum of 1000 up to 5000 mg l^{-1} as $CaCO_3$ (Holder *et al.*, 1975; Jenkins *et al.*, 1983; Hobson and Wheatley, 1993). In an anaerobic digester that is working well the TVFA:BA is normally 0.3 or less. If the ratio increases above this the system is deemed to be unstable (Carliell *et al.*, 1996). If the buffering capacity of the system is exceeded the pH may drop, leading to collapse of the anaerobic system.

Therefore parameters requiring monitoring at high loading rates include pH, BA, and VFA concentration. At lower loading rates less monitoring is required.

Due to the use of a range of chemicals containing sulphate during textile processing, high concentrations may be present in textile effluent. Concentrations of 2-4 g l^{-1} sulphate have been found to be inhibitory to anaerobic processes (Colleran *et al.*, 1998; O'Flaherty and Colleran, 1999). High sulphate concentrations are undesirable as sulphate-reducing bacteria (SRB) compete very efficiently with methanogenic bacteria for hydrogen (H_2) and other substrates (Malina, 1992) and with acetogenic bacteria for substrates such as alcohols and short-chain VFAs (Omil *et al.*, 1998). This results in the production of odoriferous hydrogen sulphide (H_2S) rather than CH_4 (Malina, 1992). One per cent of H_2S corresponds to 26 mg l^{-1} H_2S or 52 mg l^{-1} total sulphide in the liquid phase (pH 6.9, 35°C) (Speece, 1996). Concentrations of 0.1-0.8 g l^{-1} dissolved sulphide have been reported to be toxic to methanogenesis. Sulphide salts of most metals, with the exception of chromium, form insoluble precipitates. This may remove trace elements essential for methanogens (Parkin *et al.*, 1990). Sulphide concentrations of 1-25 mg l^{-1} have been cited as optimal for methanogen metabolism (Parkin *et al.*, 1990).

Methanogens require small concentrations of sodium (Na). In textile effluent the chemicals used in the dyebath are the principal source of sodium. An optimum of 0.23-0.35 g l^{-1} has been reported for organisms grown in a low salinity medium with higher optimum concentrations for organisms grown in a high salinity medium (Feijoo *et al.*, 1995). Concentrations of 3.5-5.5 g Na l^{-1} have been cited as causing moderate inhibition of methanogenesis with strong inhibition occurring at 8 g l^{-1} (Carliell *et al.*, 1996). At pH 7 sodium concentrations of 5, 10 and 14 g Na^+ l^{-1} have been found to cause 10, 50 and 100% inhibition of acetoclastic methanogenic activity respectively, with more pronounced inhibition at pHs near 8 (Rinzema *et al.*, 1988). Concentrations of 3-16 g l^{-1} caused 50% inhibition of methanisation of VFA mixtures in 3 anaerobic sludges in the absence of nutrients or other salts (Feijoo *et al.*, 1995).

1.5.2.1.1 Azo Dye Decolourisation In Anaerobic Systems.

Most dye types are at least partially degraded anaerobically, although none as readily as azo dyes (Vandevivere *et al.*, 1998). Field *et al.* (1995) reported that almost all azo dyes tested by a number of authors were decolourised by anaerobic means. Some anaerobic dye decolourisation may be due to adsorption of the dyes to the biomass but most is considered to occur by means of biological degradation. Azo dyes are reduced and hence decolourised when acting as electron acceptors for the microbial electron transport chain (Figure 1.1).

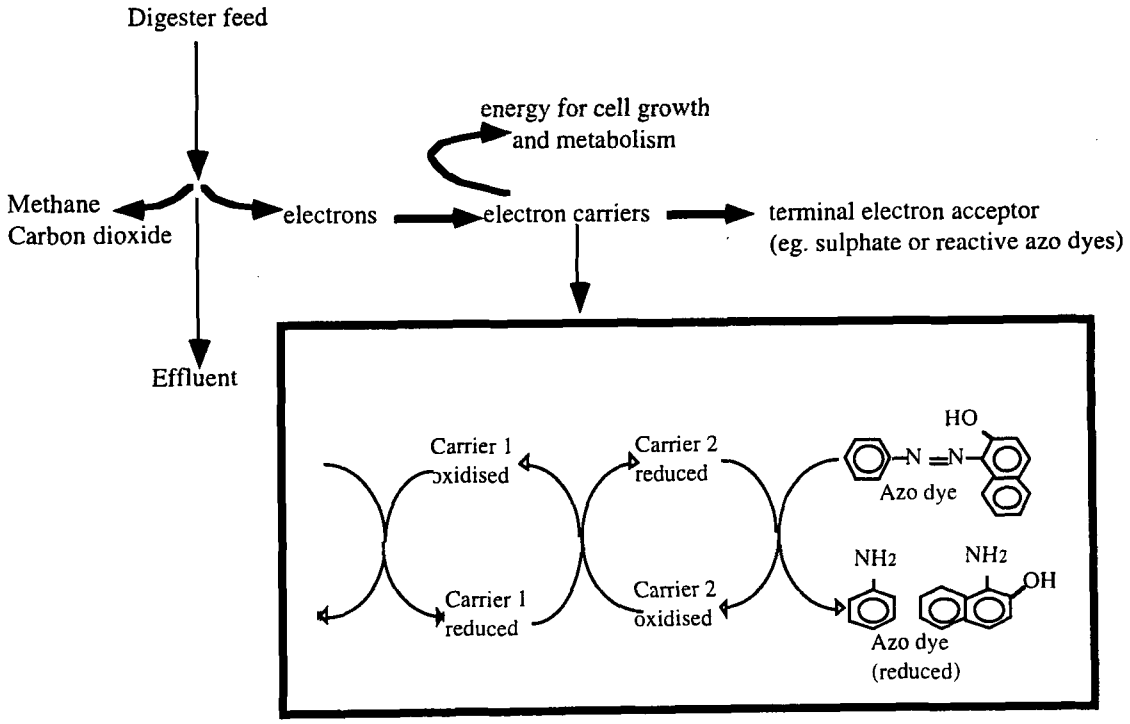


Figure 1.1 Mechanism For Anaerobic Azo Bond Reduction (After Carliell *et al.*, 1996).

A source of labile carbon is required as a source of reduction equivalents in order for dye decolourisation to occur (Carliell *et al.*, 1996). Concentrations of 5 g l^{-1} of glucose, glycerol, lactose and starch have been found to give colour removals of 82, 71, 71 and 52% respectively of 0.5 g l^{-1} Remazol Black B (Nigam *et al.*, 1996a). Minimal concentrations of co-substrate for good decolourisation have been reportedly difficult to establish. However, the low colour removal capacities reported for some systems

(Seshadri *et al.*, 1994) could be due to the low organic load of the synthetic wastewater used. Some dyes, such as azodisalicylate, can be decolourised in the absence of an additional carbon source (Razo-Flores *et al.*, 1997). In such cases the dyes provide a source of carbon and nitrogen for the anaerobic bacteria.

Reductive fission of the azo group is the first step in azo dye degradation and is readily accomplished by anaerobic micro-organisms with non-specific enzymes (Meyer, 1981; Zaoyan *et al.*, 1992; FitzGerald and Bishop, 1995). This process has two stages, the first of which generates an unstable colourless compound. This may either revert to the coloured form under oxidising conditions (Carliell *et al.*, 1994a) or become reduced to form aromatic amines. Some unstable aromatic amines have been reported to form coloured products in the absence of further treatment. This is thought to be due to spontaneous or microbially catalysed oxidation of some of the reduction products formed in anaerobic treatment (Knapp and Newby, 1995). The percentage anaerobic dye decolourisation achieved by a range of authors can be seen in Table 1.6.

Decolourisation was found to range from 0 to >99%. Some dyes in the same class gave very different results in terms of anaerobic colour removal. This may be explained in terms of dye structure. Electron-withdrawing groups on the dye molecule such as chloro, nitro and azo groups, are favourable for anaerobic reduction of dyes where nucleophilic mechanisms of degradation are prevalent (Field *et al.*, 1995). It follows that the presence of electron-donating groups, such as amino groups, are unfavourable for nucleophilic attack.

Table 1.6 Summary Of Published Work On Dye Decolourisation Under Anaerobic Conditions (After Delée *et al.*, 1998).

Author	Dye (chromogen)	% colour removal	Initial concentration	Retention time	Culture conditions and remarks
Brown and Laboureur, 1983	Mordant Blue 13 (mono azo)	83	100 mg l^{-1}	42 d	interlaboratory exercise: the presented figures are means of the reported values; batch studies in sealed bottles (0.5 l^{-1}) with an artificial test medium; inoculated with digester sludge at 35°C; mono and diazo dyestuff are readily biodegradable; polyazo, anthraquinone and the tested miscellaneous dyestuffs are less likely to be degraded.
	Mordant Black (mono azo)	77			
	Basic Red 18 (mono azo)	92			
	Acid Yellow 151 (mono azo)	88			
	Direct Red 7 (diazo)	92			
	Acid Red 114 (diazo)	62			
	Direct Blue 15 (diazo)	83			
	Direct Yellow 12 (diazo)	75			
	Reactive Black 5 (diazo)	81			
	Acid Blue 113 (diazo)	94			
	Direct Black 19 (polyazo)	51			
	Direct Black 22 (polyazo)	61			
	Reactive Blue 19 (anthraquinone)	70			
	Acid Blue 80 (anthraquinone)	7			
	Acid Blue 25 (anthraquinone)	67			
	Basic Blue 22 (anthraquinone)	62			
	Direct Yellow 11 (stilbene)	53			
	Reactive Blue 21 (phthalocyanine)	36			Reactive Blue 21 is a Cu-containing dye
	Basic Blue 3 (oxazine)	62			
	Acid Orange 3 (nitro)	62			
	Basic Yellow 28 (methine)	35			
Haug *et al.*, 1991	MY3 (C.I. 14095)(azo)	51	0.5 mmol l^{-1}	72 h	inoculated with a mixed bacterial community; 17 cm^3 test tubes; at 30°C; in the presence of 1800 mg l^{-1} glucose all azo dyes were completely metabolised, except Acid Yellow 23.
	Acid Red 27 (azo)	37			
	4-hydroxyazobenzene-4′-sulphonic acid (azo)	43			
	Acid Yellow 23 (azo)	6			
	Acid Yellow 21 (azo)	98			
Gonçalves, 1993.	Acid Red 42 (azo)	62	80 mg l^{-1}	16 h	UASB (15 l); glucose-based medium with COD 2500 mg l^{-1}; COD removal 70 %; Disperse Blue 56 made the reactor collapse
	Direct Red 80 (azo)	81	40 mg l^{-1}		
	Disperse Blue 56 (anthraquinone)	0	30 mg l^{-1}		

Table 1.6 Summary Of Published Work On Dye Decolourisation Under Anaerobic Conditions (After Delée *et al.*, 1998) cont.

Author	Dye (chromogen)	% colour removal	Initial concentration	Retention time	Culture conditions and remarks
Carliell *et al.*, 1994b	Reactive Yellow 16 (azo)	80-90	100 mg l^{-1}	6.5 h	batch studies in sealed serum bottles (0.120 l);
	Reactive Red 198a (azo)	85-90	"	2 h	assay medium consisted of 1 g l^{-1} glucose in a phosphate buffer at 32°C;
	Reactive Red 141 (azo)	85-90	"	4.5 h	inoculum was digester sludge from wastewater works receiving textile effluents;
	Reactive Blue 220 (azo)	90-95	"	1 h	*no exact concentrations given, the commercial printing dye solution was diluted
	Reactive Yellow 95 (azo)	0	1:1000*	-	1000-fold;
	Reactive Orange 12 (azo)	90-95	"	23 h	authors attributed the absence of colour removal with Reactive Yellow 95 to
	Reactive Red 218 (azo)	90-95	"	32 h	inhibitory compounds in the printing solution;
	Reactive Orange 13 (azo)	85-90	"	50 h	
	Reactive Red 24 (azo)	90-97	"	32 h	
	Reactive Brown 11 (azo)	90	"	23 h	
	Reactive Black 39 (azo)	70-75	"	5.5 h	
	Reactive Black 5 (diazo)	80-85	100 mg l^{-1}	4.5 h	
	Blue PB (metal complex)	98	1:1000*	2 h	
	Black SG (metal complex)	75-80	"	7.5 h	
	Reactive Blue 49 (anthraquinone)	7-10	"	2 h	
	Reactive Blue 38 (phthalocyanine)	40	100 mg l^{-1}	4.5 h	
	Reactive Blue 21 (phthalocyanine)	85-90	"	4.5 h	
	Reactive Blue 72 (phthalocyanine)	25-30	1:1000*	50 h	
Seshadri *et al.*, 1994	Acid Orange 7 (azo)	90	5 mg l^{-1}	24 h	labscale (2.3 l) fluidised bed reactor at 25°C;
	Acid Orange 8 (azo)	98		12 h	synthetic wastewater with 160-185 mg COD l^{-1};
	Acid Orange 10 (azo)	81		12 h	retention times below 10 h greatly reduced the colour removal;
	Acid Red 14 (azo)	86		24 h	COD removal 40%; at dye concentrations above 15 mg l^{-1} COD removal was reduced.
Carliell *et al.*, 1995	Reactive Red 121 (diazo)	various	various	various	inhibition of anaerobic bacteria occurred at dye concentrations above 100 mg l^{-1}; biomass previously exposed to the dye was more resistant to toxicity; glucose as carbon source enhanced biodegradation; the presence of nitrate inhibited decolourisation; sulphate did not have a detrimental effect; low redox potential gave rapid decolourisation.

Table 1.6 Summary Of Published Work On Dye Decolourisation Under Anaerobic Conditions (After Delée *et al.*, 1998) cont.

Author	Dye (chromogen)	% colour removal	Initial concentration	Retention time	Culture conditions and remarks
Knapp and Newby, 1995	Chlorazol Yellow (diazo)	70-85	1:10 dilution	72 h	0.200 l anaerobic reactors, inoculated with anaerobic sludge at 30°C; 10% (v/v) chemical industry effluent from the production of optical brighteners; decolourisation favoured by highly proteinaceous media.
An *et al.*, 1996	Acid Yellow 17 (azo) Basic Blue 3 (phenoxazine) Basic Red 2 (acridine)	20 72 78	40 mg l^{-1}	8-20 h	labscale UASB (4.5 l); medium was a glucose solution at 1000 mg COD l^{-1} COD removal 50-90%.
Nigam *et al*, 1996a, 1996b	Remazol Golden Yellow RNL (azo) Remazol Navy Blue GG (diazo) Remazol Red RB (diazo) Remazol Blue B (diazo) Remazol Black B (diazo) Cibracon Orange CG (n.i.) ** Cibracon Red C-2G (n.i.) Disperse Navy D2GR (n.i.) Remazol Turquoise Blue G133 (phthalocyanine)	78 80 89 76 67 79 88 68 8	500 mg l^{-1}	24 h	batch studies in sealed glass vessels, at 26°C; isolated microbial consortium *Alcaligenes faecalis* and *Commamonas acidovorans*; decolourisation dependent upon the presence of an additional carbon and energy source (yeast extract); ** n.i. = chromophore not identified. Turquoise Blue is a Cu-containing dye.
Oxspring *et al.*, 1996	Remazol Black B (diazo)	> 95	500 mg l^{-1}	48 h	labscale (0.125 l) upflow anaerobic filter with immobilised microbial consortium (12 - 20°C).
Donlon *et al.*, 1997	Mordant Orange 1 (azo)	95	100 mg l^{-1}	8 h	labscale (0.160 l) UASB at 30°C; medium was a glucose solution at 1420 mg COD l^{-1}; 86% COD removal; glucose was a better co-substrate for decolourisation than VFA; one of the azo-cleavage products (5-aminosalicylic acid) was completely mineralised to methane.
Razo-Flores *et al.*, 1997	Mordant Orange 1 (MO1)(azo)	> 99	100 mg l^{-1}	8 h	labscale (0.160 l) UASB at 30°C; medium with MO1 was a glucose solution at 1420 mg COD l^{-1}; 86% COD removal; after 217 days of operation with MO1 switched to ADS, with a glucose medium at 3000 mg COD l^{-1}; 95% COD removal; ADS was completely mineralised; after an additional 206 days of operation co-substrate feeding was stopped; complete mineralisation of ADS was observed in the absence of any other carbon source.
	Azodisalicylate (ADS)(azo)	98.8	75 mg l^{-1}	8 h	
	Azodisalicylate (azo)	88.9	75 mg l^{-1}	24h	

Dyes are not normally cytotoxic, mutagenic or carcinogenic, but the amines formed by anaerobic digestion may possess these characteristics (Chung and Stevens, 1992; Harmer *et al.*, 1992; Zaoyan *et al.*, 1992; Brown and Devito, 1993; FitzGerald and Bishop, 1995). Walker (1989) reported that amino-derivatives caused respiration inhibition effects. Bacterial luminescence tests found aromatic amines produced by anaerobic decolourisation of C.I. Reactive Violet 5 to be more toxic than the dye itself (Sosath and Libra, 1997). Amines are therefore thought to be more toxic than the original azo compounds and so further treatment is essential prior to discharge.

Aromatic amines are usually not degraded further anaerobically (Meyer, 1981; Zaoyan *et al.*, 1992; FitzGerald and Bishop, 1995) as they are quite stable (Loyd *et al.*, 1992). Therefore decolourisation is normally achieved with no elimination of chemicals from the water (Boe *et al.*, 1993). Hence dyes do not provide a source of nitrogen or carbon to anaerobic bacteria. However, the aromatic amines can be degraded by non-specific enzymes of aerobic micro-organisms, which cause hydroxylation and ring-opening (Zaoyan *et al.*, 1992; Boe *et al.*, 1993; Zissi and Lyberatos 1996). They are therefore more susceptible to aerobic degradation than the original compounds (Donlon *et al.*, 1996). Such aerobic degradation of amines means that the dyes would therefore provide a source of nitrogen and carbon for the aerobic stage. One study carried out on the aerobic biodegradability of a range of aromatic amines found they were rapidly degraded in excess of 90%. They were thus unlikely to remain in the environment for any length of time (Brown and Laboureur, 1983). Hence azo dyes may be fully degraded by a combination of anaerobic and aerobic treatment.

1.5.2.1.2 Advantages And Disadvantages Of Anaerobic Treatment.

Anaerobic treatment has a number of advantages and disadvantages as can be seen from Table 1.7.

Table 1.7 Advantages And Disadvantages Of Use Of Anaerobic Digestion In Treatment Of Textile Effluents.

Advantages	Disadvantages
High BOD and COD can be removed	BOD removal can be insufficient
Biogas is produced which can be used as a source of energy	Uneconomical in treatment of wastes with low COD
Heavy metals can be retained through sulphate reduction	Sulphates give rise to sulphide and may cause toxicity or give rise to odour
High pH effluents can be acidified	Reactor recovers more slowly after a toxic shock
No foaming	Susceptible to sudden changes in operating conditions and substrate load
High effluent temperatures can be favourable	Reactors must normally be heated
Can handle complex heterogeneous substrates even at low temperatures	Acclimated bacterial culture is required
Degradation of refractory organics can be initiated	Dyes and other refractory compounds are not mineralised
Small amount of biomass is generated	
The waste is stabilised to a high degree	

It is economical to treat wastes with CODs exceeding 3000-4000 mg l^{-1} as surplus energy may then be generated and used to heat the system (Olthof and Oleszkiewicz, 1982; Thampi, 1998). It has been reported that wastes containing up to 30,000 mg l^{-1} COD may be treated anaerobically (Willmott *et al.*, 1998). Good contact between the bacterial population and the substrate is required and the pH must be maintained at approximately 7. Acclimation may enable the micro-organisms to withstand concentrations exceeding the unacclimated threshold level of recalcitrant or toxic chemicals as demonstrated by Olthof and Oleszkiewicz (1982) with formaldehyde and phenol.

Anaerobic treatment has a lower rate of biomass growth than aerobic treatment. Therefore a longer biomass retention time is required, although this does not necessarily affect the HRT (Olthof and Oleszkiewicz, 1982). The lower growth rate means less nutrients are required than in aerobic systems (Gray, 1989), which is advantageous, and that the system recovers more slowly after a toxic shock (Olthof and Oleszkiewicz, 1982), which is not. About 5% of the BOD is converted into new biomass (Tang *et al.*, 1995), far less than that produced by aerobic treatment methods. In anaerobic processes the F:M ratio is typically in the range of 0.5-1 g BOD g^{-1} V(S)S d^{-1}, exceeding those obtained in aerobic systems (Speece, 1996). The sludge loading rate (B_x) is described in terms of g COD g^{-1} VSS d^{-1} and therefore is higher than the F:M. An adequate supply of nutrients must be provided with the correct C:N:P (Holder *et al.*, 1975). Forster (1991) cited a COD:N:P of <350:5:1 for good operation of anaerobic systems. Speece (1996) cited a COD:N:P of 350:7:1 for highly loaded systems and 1000:7:1 for lightly loaded systems. Laing (1991) cited a BOD:N:P of 100:5:1 for biological treatment of wastes generally. Further treatment may be required subsequent to anaerobic digestion to produce an effluent complying with discharge standards. However, the main barrier to use of anaerobic treatment of industrial waste is said to be the lack of experience with the process (Switzenbaum, 1995).

1.5.2.2 Aerobic Treatment.

Dyes do not inhibit activated sludge in the concentrations normally found in wastewater (Durig, 1981). However, the enzymes involved are so specific that only certain dyes are degraded (Zissi and Lyberatos 1996). The efficiency of activated sludge processes in treatment of textile finishing wastewater can be increased by acclimatisation of the bacteria to the chemicals contained in the waste (Groff, 1991). This may take a long time, however, as Field *et al.* (1995) cited 100 to 400 generations for adaptation to the cleavage of carboxylated azo dyes. Partial treatment of dyes by means of conventional aerobic treatment is attributed to precipitation of insoluble dyes (such as disperse, vat, sulphur, and azoic dyes) in the primary settling tank, or adsorption of soluble dyes to the sludge (Correia *et al.*, 1994). Other potential methods of dye removal in activated sludge

treatment include chemical transformation, photodegradation and air stripping although these processes have been proved unlikely to occur (Shaul *et al.*, 1986).

The removal of dyes by sorption during biological treatment is referred to as bioelimination (Laing, 1991). The adsorbed dyes are removed during sludge disposal (Pierce, 1994). Sorption is influenced by factors such as pH but the extent of removal varies from dye to dye (Laing, 1991). Dyestuffs with a higher affinity for the sludge are sorped faster and therefore fractions of total removal for different dyes after a given time vary greatly (Mittal and Gupta, 1995). If dye is strongly adsorbed the adsorption sites become occupied rapidly and are thus unavailable for further adsorption. Renewal of these sites occurs through desorption, biodegradation or generation of new sludge flocs. However, desorption leads to resolubilisation of colour, aerobic biodegradation of dyes tends to be slow and in normal sludges the generation of new flocs is too slow to permit adequate removal of dyes (Churchley, 1994). It is thought that at very low dye concentrations there is an almost linear relationship between dye concentration and the quantity of dye adsorbed by activated sludge (Hitz *et al.*, 1978).

Laing (1991) reported that colour removal from textile effluents by activated sludge processes ranged from 10 to 80%, typically being below 50%. Shaul *et al.* (1986) carried out a study on seven acid azo dyes and found that both adsorption and biodegradation played a role in colour removal, varying with each dye. Pagga and Brown (1986) carried out tests on 87 dyestuffs and found that colour was removed by means of adsorption but little biodegradation occurred. Reactive and acid dyes exhibit very low adsorption and are highly water soluble; disperse dyes adsorb in the medium to high range; and basic and direct dyes exhibit a high degree of adsorption (Hitz *et al.*, 1978; Pierce, 1994; Willmott *et al.*, 1998). A number of authors reported elimination of reactive dyes by adsorption onto sludge ranging from 0-30% (Sewekow 1993; Holme and Thornton, 1994; Pierce, 1994; Waters, 1995). The poor adsorption of reactive dyes is not related either to the number of sulphonic acid groups on the molecules or to the ease of dye hydrolysis (Hitz *et al.*, 1978). It is aggravated by the high water solubility and low fixation rate of reactive dyes. It can be seen therefore that aerobic treatment alone is ineffective in treatment of effluent from cotton processing.

High-rate aerobic systems must be continuously aerated, an energy-intensive process, to ensure survival of the bacteria. The activity of most aerobic micro-organisms is reported to be inhibited below 7°C and above 36°C (Gray, 1989). Most activated sludge tanks are maintained at ambient temperature in temperate climates. Aerobic treatment is suitable for effluents with a COD of 40-4000 mg l^{-1} (Thampi, 1998). It has been reported that aerobic systems are limited by oxygen transfer to wastes of less than 3000-5000 mg COD l^{-1} (Olthof and Oleszkiewicz, 1982). However, according to Lin and Peng (1995), activated sludge processes are inadequate for treatment when the COD of textile effluent exceeds 1200 mg l^{-1}. Aerobic treatment produces about ten times more biomass than anaerobic treatment as 40-60% of the COD entering the system is converted to sludge (Olthof and Oleszkiewicz, 1982; Crites and Tchobanoglous, 1998). Disposal of this is expensive.

Typical mixed liquor suspended solids (MLSS) concentrations for completely mixed activated sludge systems are cited as ranging from 0.8 to 6.5 g l^{-1}. Between 40 and 85% of the MLSS is typically volatile and the mean sludge age is 3-8 days, with a range of 0.75-15 days. Typical F:M ratios are cited as ranging from 0.1 to 0.5 g BOD g^{-1} biomass d^{-1} (Speece, 1996). Therefore aerobic F:M ratios are lower than those achieved in anaerobic systems which means that anaerobic systems can exhibit higher volumetric loading rates. At high F:M ratios high growth is achieved resulting in growth of filamentous micro-organisms, which do not settle well. Therefore substances such as starch and acetic acid, which have high BOD, may lead to bulking problems. At low F:M ratios bacterial growth cannot be sustained and endogenous metabolism or auto-oxidation occurs where weaker cells die and become a source of food for the remaining cells. The non-biodegradable cell capsules and viable cells then form a pin-point floc, which does not settle properly. Therefore a balance between the two extremes must be achieved. Nutrients may need to be added to industrial effluents to enable biological treatment to be used in their treatment (Laing, 1991). A minimum BOD:N:P of 100:2:0.3 has been recommended for activated sludge systems operating at low F:M ratios (British Textile Technology Group, 1996). A ratio of 100:5:1 has been cited for activated sludge treatment of wastes in general (Shastry and Thambirajah, 1995; Sapari, 1996).

1.5.2.3 Combined Treatments.

Shelley *et al.* (1976) stated that a combination of more than one process was normally required to treat textile wastes sufficiently prior to discharge to receiving waters. Sapari (1996) found that pre-treatment of textile wastewater from desizing, scouring, bleaching and dyeing processes was required prior to biological treatment due to the inhibitory nature of some of the chemicals present to the activated sludge process.

Coagulation combined with activated sludge treatment has been investigated by a number of authors including Shelley *et al.* (1976), Hamza and Hamoda (1980) and Altinbas *et al.* (1995). Combinations of ozonation, coagulation and activated sludge treatment (Lin and Lin, 1993; Lin and Liu, 1994); and coagulation, biological oxidation, pressurised air flotation and filtration have also been tested (Groff, 1991). Literature revealed little work on coagulation/flocculation combined with anaerobic-aerobic treatment. However, anaerobic treatment of chemically coagulated organic sludge found the digestibility of a range of organic substrates was decreased; digesters were destabilised; a lower methane yield was obtained; and a greater quantity of less stable solids was generated for disposal. These effects were not attributable to toxicity, increased solids concentration or phosphate immobilisation (Dentel and Gossett 1982). Therefore coagulation prior to anaerobic treatment may be problematic.

Biological treatments are often combined with physical/chemical methods and combinations of biological treatments have also been used. Combinations of anaerobic and aerobic treatment have been carried out by a number of authors. An *et al.* (1996) treated acid and basic dye solutions and dye manufacture wastewater with an upflow anaerobic sludge blanket digester (UASB) and a semi-continuous activated sludge stage. Zhu *et al.* (1994) treated dye manufacturing waste and glucose with a UASB and an activated sludge process. Zaoyan *et al.* (1992) treated textile wastewater with anaerobic and aerobic rotating biological contactors (RBCs). Boe *et al.* (1993) used anaerobic reactors with a continuously mixed activated sludge stage to treat effluent from the primary clarifier in a STW, composed of 75% textile wastewater and 25% domestic and industrial waste. Loyd *et al.* (1992) carried out anaerobic and aerobic treatment of textile

effluent and of dye combined with municipal wastewater. Sequencing batch reactors (SBRs) were used for the anaerobic stage together with a continuous activated sludge stage. Anaerobic treatment was found to decolourise azo dye wastewater significantly with little accompanying biodegradation while aerobic treatment significantly biodegraded the wastewater but provided little decolourisation. Anaerobic pre-treatment enhanced subsequent aerobic biodegradation. Other authors who investigated anaerobic-aerobic treatment include Seshadri *et al.*, (1994) and FitzGerald and Bishop (1995). A sequential anaerobic-aerobic system for treatment of textile effluent is in operation at a major new dyehouse in Hong Kong (Easton, 1995). However, although information on anaerobic-aerobic treatment of textile effluent and dyes is available, literature revealed few studies on combined anaerobic-aerobic treatment of either reactive dyes or effluent from cotton processing.

1.6 Project Objectives.

Due to the large volumes of textile effluent produced world-wide, and the increasing impact of new legislation on textile producers, the treatment of such effluent was examined in this project. Dyeing of cotton with reactive dyes produces highly coloured effluent. Therefore this effluent type was selected for study. Simulated waste was used in this project due to the absence of a local source of effluent and to eliminate the variability associated with real wastes. It was planned to use a simple simulated textile effluent containing the principal compounds normally found in textile effluent and a dye of known structure. The great variation in dye quantities and types typically present was to be excluded. The work had the following aims:

- To generate a simulated effluent that was representative of real cotton processing effluent while maintaining a simple composition.

- To investigate the possibility of using coagulation/flocculation as a pre-treatment process, as there was little information available on use of these treatments prior to anaerobic digestion.

- To assess an inclined tubular digester (ITD) and an upflow anaerobic sludge blanket digester (UASB) to determine which was the most suitable for anaerobic treatment of this waste type. The ITD was initially selected when it was envisaged that coagulation/flocculation treatment of the incoming textile wastewater would give a concentrated waste stream for anaerobic treatment. Some applications of ITDs on a laboratory scale have been reported (Section 4.1). However, the information available on these digesters was limited and none was found on their use with this type of waste. Prior studies (Section 4.1) carried out variously on dyes, textile effluent and dye manufacture wastewater, found that colour and COD removal could be obtained using UASBs. However, little information on UASBs in treatment of effluent from cotton processing, real or simulated, or reactive dyes was discovered. Few authors have used combined anaerobic-aerobic treatment in treatment of simulated textile effluent.

- To evaluate the application of a combined anaerobic-aerobic treatment system to the treatment of simulated textile effluent.

- To evaluate the effect of anaerobic and aerobic treatment on colour and COD removal.

- To establish operating parameters for the anaerobic and aerobic stages treating different simulated textile effluent compositions.

- To investigate the relationship between dye and co-substrate (starch) by altering the concentrations of the two substances in different experiments. This was done to determine whether addition of a carbon source when high dye concentrations were present in effluent would achieve better decolourisation.

- To determine whether aromatic amines were produced by anaerobic treatment and hence clarify whether anaerobic colour removal was achieved by adsorption or dye degradation.

- To discover whether such amines, if produced, were removed by means of aerobic treatment.

CHAPTER TWO - MATERIALS AND METHODS.

2.1 Introduction.

This Chapter reviews the anaerobic and aerobic reactors used in this project and the methods of analysis used for experiments. The Simulated Textile Effluent (STE) used to feed the reactors is discussed in detail in Chapter 3. It contained reactive azo dye, starch, acetic acid and salt, in addition to nutrients and trace elements. Dye concentrations ranging from 0.075-1.5 g l^{-1} were tested, while starch concentrations ranged from 0.95-3.8 g l^{-1}. This enabled a range of starch:dye ratios to be tested and the effect of each component on biological treatment to be compared. The STE could be made up either at the concentration at which it was to be fed to the reactors (days 1-184, Experiment 1) or as a concentrate (day 184, Experiment 1, onwards). Different strength concentrates were used with different Experiments. Problems were experienced with coagulation of starch when using a x30 concentrate during Experiment 3, and therefore a x10 or x15 concentrate was used thereafter. When a concentrate was used the STE was diluted with water containing sodium bicarbonate. This water was stored underneath the bench in a covered 200 l tank. When a concentrate was not used bicarbonate was added directly to the STE.

Four Experiments were carried out. Experiments 3 and 4 comprised a number of shorter Experiments, referred to as Expts. In these Expts the concentrations of starch and dye were varied.

2.2 Reactors.

The reactors used were an Inclined Tubular Digester (ITD), a 5 l Upflow Anaerobic Sludge Blanket digester (UASB), a 30 l UASB, an activated sludge tank and a settling tank. All vessels were made out of perspex and were tested for water leaks by filling them with water and leaving them to stand overnight. Any loss of water meant there was a leak as losses due to evaporation were minimal. The UASBs and aerobic vessels had

water jackets surrounding the main body of the reactors. The water jacket and main compartment were leak-tested separately.

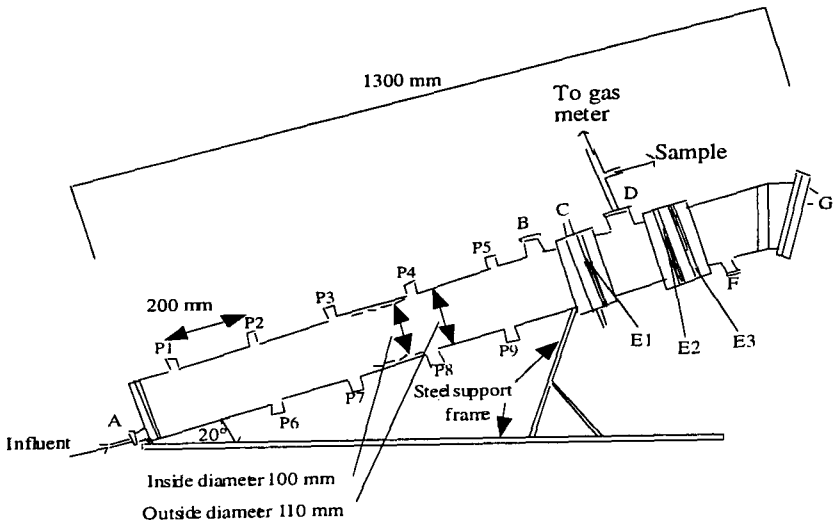
The anaerobic reactors were then checked for gas leaks by filling them with water and sealing all the ports. Nitrogen gas was pumped into the top of the reactor through the gas sampling port at the top of the reactor at a low pressure. A soap solution was placed around all ports, joints and other possible locations of leaks such as pH probe ports. Any escape of gas resulted in the production of bubbles. A manometer was also attached to the anaerobic digesters to monitor gas leaks.

All pumps used in this series of experiments were Watson-Marlow (UK) pumps. Silicone rubber tubing was used for transfer of liquids, except in the pump heads. In the pump heads marprene double manifold tubing was used to pump the STE concentrate. Watson-Marlow Marprene II tubing was used with all other pump heads unless specified. The STE concentrate and dilution water were pumped at appropriate rates into a common feed line, and mixing occurred in-line. A T-joint was built into the main feed line to enable extraction of suitably diluted influent samples for analysis.

Both UASBs had a built-in water jacket. In the ITD a water jacket was improvised by covering the reactor with coils of silicone rubber tubing through which water was pumped. The water jackets were attached to Grant FH15 flow heaters (BDH, UK) which pumped heated water through the tubing. Water coming from the reactor was displaced into a ballast bottle to eliminate air bubbles, which could cause the flow heater to fail, and to compensate for the evaporation of water over time. All anaerobic digesters were maintained at approximately 35°C thoughout the series of experiments. Therefore the mesophilic bacteria were used (Section 1.5.2.1). All reactors used here are discussed in more detail in Sections 2.2.1-2.2.4.

2.2.1 Inclined Tubular Digester (ITD).

Inclined Tubular Digesters (ITDs) minimise the gas/liquid surface area where crusts and scum can form and no mixing is required. They retain biomass by means of baffles or weirs. The inclination of the digester increases the sludge retention time (Floyd, 1984). Chapman (1986) found greatest stability at 20° inclination due to more uniform temperature distribution from mixing by biogas and convection currents. The gas yields per unit of VS added usually exceed those from comparable CSTRs due to the increased solids' retention. If the ITD were to behave as a plug flow reactor, then wastewater added at one end of the tube would be inoculated and pass along the tube without mixing while digestion occurred. However, true plug-flow is not attained in ITDs for a variety of reasons: friction on the tube walls can induce turbulence; biogas produced by digestion mixes the contents; and heating of the reactor may give rise to thermal movements. The ITD can be seen in Figure 2.1.



A	Feeding port	F	Effluent sample port
B	On-line pH port	G	Effluent outlet
C	Gas sampling port (minor)	P1-P5	Sample acquisition ports
D	Gas sampling port (major)	P6,P7,P9	Sludge recycling ports
E1,E2,E3	Weirs	P8	On-line temperature port

Figure 2.1 Schematic Diagram Of The Inclined Tubular Digester.

The STE was pumped into port A at the lower end of the ITD. Ports were located at 200 mm intervals. Ports P1 to P5 facilitated the removal of samples, which were analysed to assess the efficiency and buffering of the anaerobic reactor. Ports P6, P7 and

P9 were placed to enable sludge removal and recycling if required. A temperature probe was sealed into P8 using silicone multi-purpose sealant to facilitate on-line temperature measurements. The ports that were not in use were clamped in order to seal them. Three weirs were present towards the head of the reactor to retain solids and eliminate the requirement for a subsequent settling tank and sludge recycle. They also increased the gas pressure at the head of the reactor ensuring the gas was forced under pressure from the two connected gas ports to a Drexel bottle. Joints were sealed with 3 mm o-rings and silicone grease. The active reactor volume from which gas was produced was 8.5 l. There was an additional 0.6 l volume after E3 that contained only a small amount of settled biomass. Effluent exited through this area. Samples were initially taken from port 2 for determination of bicarbonate alkalinity (BA) and volatile fatty acids (VFAs). When a pH electrode was put on-line at Port B on day 36, Experiment 1, these samples were replaced by samples from Port 5.

2.2.2 Upflow Anaerobic Sludge Blanket Digesters (UASBs).

Upflow Anaerobic Sludge Blanket digesters (UASBs) are the most commonly used high-rate anaerobic system (Wu *et al.*, 1992; Schmidt and Ahring, 1996) and have been used to treat a range of industrial wastes. They are designed to maintain high bacterial populations thus allowing digestion of wastes at shortened hydraulic retention times (McCarty, 1982) as the sludge retention time is almost independent of the HRT (Schmidt and Ahring, 1996). Essentially the UASB can be divided into four sections: a granular sludge bed at the bottom of the reactor, a fluidised zone or sludge blanket, a 3-phase separator and a settling region. Wastewater is pumped in at the bottom and passes up through the sludge bed. Most COD removal occurs in this layer. Due to the resulting biogas production a fluidised zone is formed where further biodegradation can occur. In this region the granules rise up through the reactor carried by the biogas until they reach the separator. Above the separator is a quiet zone, which enables the bacterial granules to flocculate. Granules with good settleability return to the sludge bed while flocculated and dispersed bacteria wash out of the reactor with the effluent.

UASBs are generally used for wastewaters with a low concentration of suspended solids (Angenent and Dague, 1995). They have a number of advantages over other anaerobic systems: higher loading rates can be achieved and therefore smaller reactor volumes can be used; no subsequent sedimentation tanks are required; and no artificial mixing of the reactor is required (Wentzel *et al.*, 1994) - in fact, mechanical mixing may damage the structure of the granules (Ross and Smollen, 1982). However, at low sludge loading rates the contents of UASBs are not adequately mixed. Therefore a loading rate sufficiently high to give rise to good mixing is required. The system requires bacterial granules that settle well and that can be mixed by circulation of the biogas produced. Granular sludge has a number of advantages over flocculant biomass: it is better retained within the reactor due to its superior settleability; it has higher specific methanogenic activity; and the methanogenic activity is maintained during less favourable conditions due to the higher internal pH of the granules (Angenent and Dague, 1995). The 5 l and 30 l UASB used in this project are detailed below.

2.2.2.1 5 L UASB.

Influent entered at the bottom of the UASB and was dispersed through a T-piece at the centre of the reactor's base (Figure 2.2). The base was removable, as was the upper section containing the 3-phase separator. The separator was an arrangement of two funnels, one inside the other. Gas was collected at the top of the reactor and passed to a Drexel bottle and from there to a gas meter. Two ports were present at the top of the reactor for measuring pH on-line and taking samples respectively. Joints were sealed with 4 mm o-rings and silicone grease.

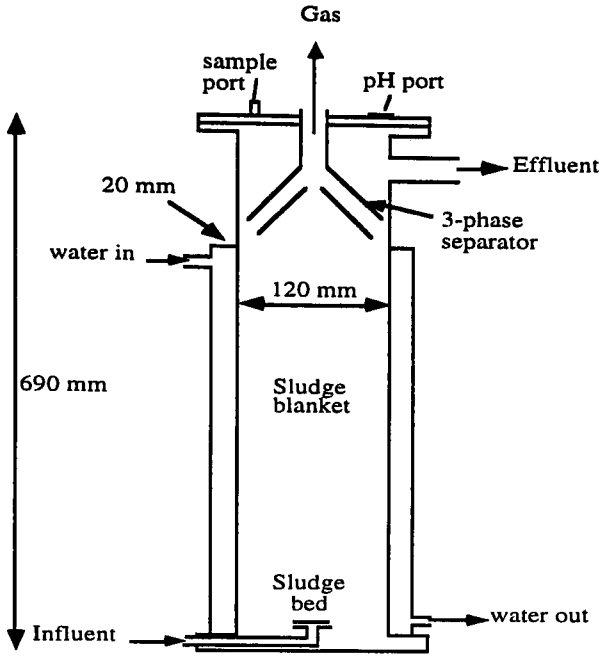


Figure 2.2 5 l UASB.

2.2.2.2 30 L UASB.

The 30 l UASB (Figure 2.3) had two influent ports, each of which led to a cross-shaped outlet at the base of the reactor. This ensured more even distribution of STE over the base of the reactor and meant that there was less likelihood of a serious blockage occurring at this point. The base and upper unit containing the 3-phase separator were removable. The 3-phase separator consisted of two pieces of perspex, shaped like segments of an orange, each extending across approximately three quarters of the width of the reactor. This separator was used as it was thought it would be more effective than the funnel arrangement used previously. An on-line pH probe was installed in one of the ports in the lid while the other ports enabled samples to be taken and various instruments to be attached. Sponge cord (8 mm) was glued in the recesses at the joints and smeared with silicone grease in order to provide air-tight seals.

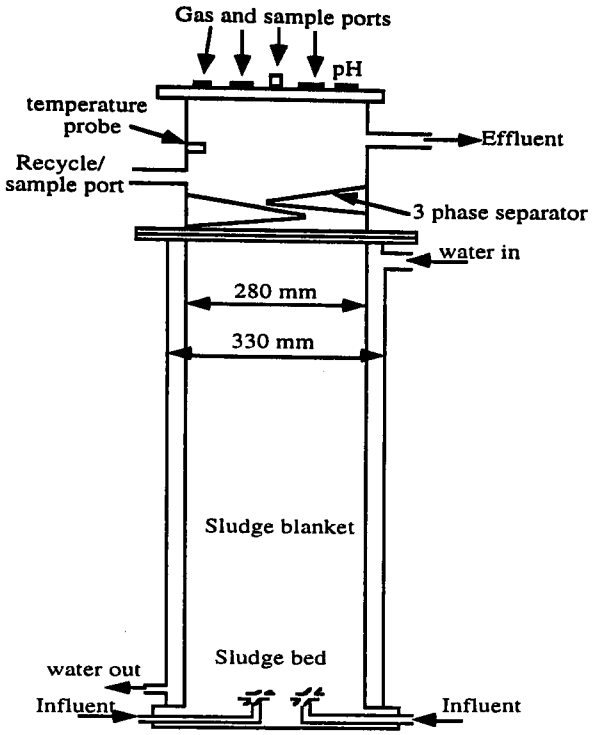


Figure 2.3 30 l UASB.

2.2.3 Aerobic Stage.

Effluent from the anaerobic digesters was collected in an Erlenmeyer flask, which acted as a mixing vessel. In the case of the UASBs, liquid was displaced into this vessel through a funnel system attached to plumbing pipe, available from any hardware store. This in turn led to a funnel at the mouth of the flask. Liquid from the ITD was collected in a funnel attached to silicone rubber tubing leading to the mixing vessel. Inside the mixing vessel was a tube, attached to a pump, reaching to the bottom of the vessel. This removed liquid and displaced it to the aerobic stage. During periods when the aerobic stage was not in use, anaerobic effluent was diverted to drain through the arm of the Erlenmeyer flask, which was attached to a drainage system. This was achieved by switching off the pump transferring the liquid to the aerobic stage.

The aerobic reactor was a cylindrical vessel 410 mm in height and 300 mm in width resting on a square base of 360 mm length. It had a water jacket, which was not used in this series of experiments as there were no extremes of temperature in the laboratory. The level of liquid in the aerobic vessel was controlled by altering the depth of a tube

within the reactor. This tube was attached to a pump and removed liquid rising above the set level. The depth was set at 10 l of liquid in Experiment 1, and at 20 l in Experiments 3 and 4. The tubes displacing liquid from the mixing vessel to the aerobic stage and from the aerobic stage to the settler were operated at speeds in excess of the minimum required. This ensured that no build-up of liquid occurred at any stage should output from the anaerobic digesters vary for any reason.

The activated sludge tank was aerated by two Capex L2C air compressors (BDH, UK) attached to three air stones located inside the vessel. From day 21, Experiment 3 the pH of the aerobic stage was controlled in an on-off manner by a neural network (Section 1.1) with a high set-point of pH 7.2, causing addition of 1M HCl. In Experiment 4 one air compressor was controlled by a neural network in an on-off manner with a lower dissolved oxygen set-point of 3 mg l^{-1}. This enabled extra oxygen to be supplied to the activated sludge stage when the load was high. In Expt 4.5 the aerobic stage was fed with a concentrated (x30) supplement of OECD synthetic sewage (OECD, 1981), stored in a refrigerator for 7 days maximum. This concentrate contained 4.8 g l^{-1} of peptone; 3.3 g l^{-1} meat extract; 0.9 g l^{-1} urea; 0.21 g l^{-1} sodium chloride; 0.12 g l^{-1} calcium chloride dihydrate; 0.06 g l^{-1} magnesium sulphate heptahydrate; and 0.84 g l^{-1} di-potassium hydrogen orthophosphate. It was mixed with UASB effluent before entering the aerobic system. This replicated a system fed partly with domestic sewage.

2.2.4 Settler.

The settler contained 3.75 l of liquid. Liquid was pumped from the aerobic stage to the settler, entering at the top of the vessel. Effluent was displaced through the effluent port to drain. A sludge recycle port was located at the bottom of the reactor. In Experiment 1 solids were recycled every eight hours, with the aid of an RS 139-710 7 day timer (RS, UK). In Expts 3.0-3.4 recycling was carried out every four hours and in Experiment 4 and Expt 3.5 solids were recycled continually. A slow stirrer was used with the settler, consisting of a 1 rpm pump connected to a stainless steel rod with two strips of rubber

attached. The rubber brushed against the upper and lower sections of the funnel-shaped base of the settler and enhanced settling of the solids (Figure 2.4).

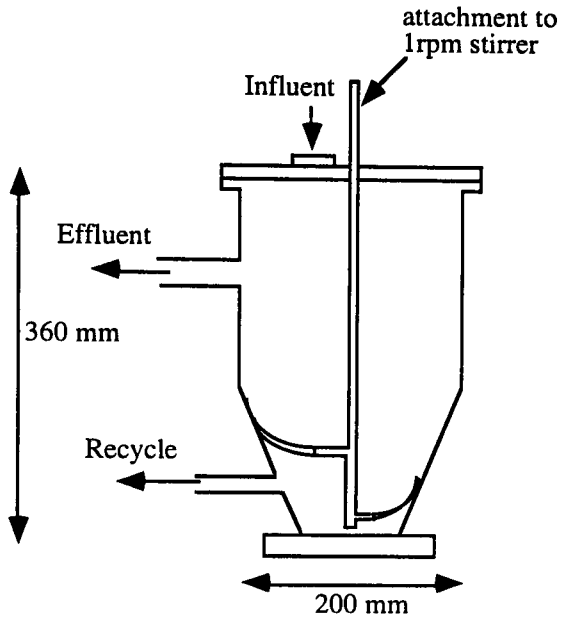


Figure 2.4 Settler.

2.3 Hydrolysis.

The STE contained starch and reactive azo dye (Section 2.1) which were hydrolysed to represent the form in which these substances are usually found in textile effluent.

2.3.1 Starch.

The method of starch hydrolysis was based on information obtained from IST (Portugal), who based it on desizing methods used in industry (Hoechst Aktiengeselschaft, 1991). To make up 1 l of solution, 40 g of NaOH pellets was dissolved in approximately 200 ml tap water in a beaker. One hundred grams of starch (in this case, Tissalys 150) was dissolved in approximately 500 ml of tap water in a separate container. The NaOH solution was added to the starch solution and the resulting mixture made up to volume with tap water and stirred. The starch stock was left to hydrolyse on the bench-top overnight as specified by IST. It was then ready for use in the simulated textile effluent.

2.3.2 Dye.

Dye was hydrolysed using a method recommended by IST (Lisbon, Portugal) who based it on information obtained from Dystar (Portugal). In order to make up one litre of dye stock at the 5% saturation recommended by the dye supplier (BASF, Manchester, UK), 50 g of dye powder was dissolved in about 900 ml of water. The solution was adjusted to pH 12 using 1.0 M NaOH (approximately 10 ml l^{-1} dye) and made up to volume. The stock solution was then heated at 80°C in an oven for 1.5 hours. The solution was allowed to cool and then made up to volume once more to compensate for the small quantity of water lost by evaporation during the heating process. It was then kept refrigerated until required.

2.4 Bicarbonate Alkalinity.

Bicarbonate alkalinity (BA) buffers anaerobic systems against changes in pH. Alkalinity is measured as mg $CaCO_3$ per litre to a specified pH for historic reasons. Bicarbonate alkalinity was measured by titration with standardised 0.05 M HCl to pH 5.75 as proposed by Jenkins *et al.* (1983). This method was deemed by its authors to measure BA with a 'high degree of confidence,' as titration to this end point reflected mainly alkalinity due to the presence of bicarbonate. Even at high VFA concentrations it was reported that an accurate estimate of bicarbonate alkalinity could be obtained. Titration of 25 ml aliquots was carried out at room temperature immediately after collection once the initial pH had been noted. A Mettler Toledo 340 pH meter (Switzerland) with a glass electrode was used. Samples were stirred with a magnetic stirrer while additions were made. The bicarbonate alkalinity was calculated as follows:

$$\text{BA mg } CaCO_3\ l^{-1} = \frac{A \times M}{\text{ml sample}} \times 1.25 \times \frac{500}{0.01}$$

A-ml of standard acid used

M-molarity of the acid

2.5 Chromatography.

Two types of chromatography were used here: High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). In HPLC a liquid mobile phase transports a sample through a column. Samples are separated in the column and elute at characteristic times under specified conditions. The components can be identified by comparison with a standard. This method was used to determine nutrient concentrations of liquid samples and investigate aromatic amine generation and degradation. In gas chromatography samples are injected either as liquids, which are then vapourised, or gases. They are then carried through the column by a carrier gas and elute at characteristic times under named conditions, as in HPLC. Gas chromatography was carried out to determine VFA concentrations and percentage carbon dioxide and methane in liquid and gaseous samples respectively.

2.5.1 High Performance Liquid Chromatography (HPLC).

2.5.1.1 Ion Exchange Chromatography.

In ion exchange HPLC the column is filled with ion exchange resin and separations are determined by the strength of the interactions between solute ions and the exchange sites on the resin (Lindsay, 1992). Analysis for cations and anions was carried out using an LC 20 Chromatography enclosure, a Dionex CD20 Conductivity Detector, and GP40 Gradient pump. An Ionpac AS4A-SC (4x250 mm) analytical column (Dionex, UK) was used for analysis of anions. The conditions used were those specified by the manufacturer (Dionex, UK) (i.e., eluent: 1.7 mM sodium bicarbonate, 1.8 mM sodium carbonate; Flow rate: 2 ml min^{-1}). An Ionpac CS12A (4x250 mm) analytical column (Dionex, UK) was used for cation analysis under conditions specified by the manufacturer (i.e. eluent: 11 mM H_2SO_4; Flow rate: 1 ml min^{-1}). Guard columns were used prior to these columns. Standards used can be seen in Table 2.1.

The machine was calibrated prior to analysis of samples. One ml of samples and standards was injected through a 0.2 μm filter to remove any solids that might be present. Samples were repeated at different dilutions if necessary to obtain peaks within the area of the graph. The concentration at the lowest dilution of sample to provide an entire peak was selected as the true concentration of each nutrient.

Table 2.1 Concentration Of Nutrients In Standards.

Anions	Conc. (mg l^{-1})	Cations	Conc. (mg l^{-1})
Fluoride	2.0	Lithium	0.5
Chloride	3.0	Sodium	2.0
Nitrate	10.0	Ammonium	2.5
Phosphate	15.0	Potassium	5.0
Sulphate	15.0	Calcium	5.0

conc. - concentration

2.5.1.2 HPLC-UV Analysis.

HPLC-UV analysis was carried out by IRSA (Italy) on samples arising from the work described here as described in O'Neill *et al.* (1999b). A UV detector was used in place of a conductivity detector. UV detectors detect only substances absorbing UV radiation, such as aromatics (Lindsay, 1992). This analysis was done to determine whether aromatic amines were produced by means of anaerobic degradation of the dye in simulated textile effluent, and if so, whether the amines were then removed aerobically. Total Organic Nitrogen (TON) content (American Public Health Association, 1989) was measured by IRSA (Italy) for comparison with the results of analysis for amines.

Samples were filtered through GF/C filter-paper using a buchner funnel and adjusted to a pH below 2 with H_2SO_4 prior to sending them for analysis. Samples were pre-treated prior to HPLC analysis, in order to purify and concentrate them. This was done as follows: 10 ml of sample were aspirated through a CHROMABOND HR/P cartridge (MACHEREY-NAGEL Inc., Easton, PA, USA) conditioned by consecutively feeding 2 ml each of methanol, acetonitrile and 10^{-5} M NaOH. The cartridge was then washed with

2 ml of distilled water, dried under vacuum and eluted by 3 x 1 ml methanol/acetonitrile 1/1 v/v.

A Varian 9012 gradient pump equipped with a C-18 chromatographic column and a Varian 2550 UV detector (Varian, Palo Alto, CA, USA) were used. Two different C-18 reversed phase chromatographic columns (3x250 mm, stationary phase particle size: 5 mm) were used: 250/3 NUCLEOSIL 100-5 C18 AB (MACHEREY-NAGEL Inc., Easton, PA, USA) and SUPELCOSIL LC-18 (SUPELCO, Bellefonte, PA, USA). The latter is the most widely used chromatographic column for non-specific HPLC detection of UV-absorbing compounds. The NUCLEOSIL C18 column is reported by the manufacturers to be suitable for the detection of aromatic amines formed during azo dye degradation and particularly appropriate for the specific detection of sixteen aromatic amines commonly formed during this process.

When the NUCLEOSIL column was used, the experimental conditions were those specified by the manufacturer for analysing aromatic amines (i.e. eluents: acetonitrile (A), 5 mM pH 7 phosphate buffer solution (B); gradient: A/B (5/95) in 35 min to A/B (85/15); flow rate: 0.6 ml min^{-1}; l: 282 nm). Different experimental conditions were fixed when the SUPELCO column was used (i.e. eluents: acetonitrile (A), 5 mM pH 7 phosphate buffer solution (B); gradient: A/B (70/30) in 40 min to A/B (30/70); flow rate: 0.6 ml min^{-1}; l 254 nm). The gradient involved changing the composition of the eluents in order to elute the components of the sample in a reasonable time when the range of eluting times is great.

2.5.2 Volatile Fatty Acids (VFAs).

Volatile fatty acids were measured on a Varian Star 3400CX analyser with a 6 ft x 4 mm glass column of 15% SP1220/1% H_3PO_4 on 100/120 Chromosorb W/AW (SUPELCO Inc., USA) with a flame ionisation detector (FID), connected to an autosampler and a GC Star Workstation (Varian Associates Inc., USA). The FID detects gases which ionise when they burn (Fowlis, 1995). The system set-up and method were as recommended

by the manufacturers (i.e. carrier gas: Nitrogen; flow rate through column: 20-60 ml min^{-1}; flow rate of hydrogen and air to FID: 30 and 300 ml min^{-1} respectively). The column, injector and detector were at temperatures of 130°C, 135°C and 155°C respectively. A standard stock was prepared (Table 2.2) and standards were made up consisting of 100%, 75%, 50%, 25% and 10% of the stock solution.

Table 2.2 Concentration Of VFAs In The Standard Stock.

Acid	Concentration (mg l^{-1})
acetic acid	1000
propionic acid	400
i-butyric	200
n-butyric acid	200
i-valeric	100
n-valeric acid	100

Samples were prepared in a manner based on that used by Peck *et al.* (1986). Five ml of sample or standard were placed in glass 13x120 mm tubes with screw-on solvent-resistant lids. Then 0.75 ml of orthophosphoric acid was added in a fume cupboard. This was followed by 5 ml of chromatography grade diethyl ether, containing 0.1 ml l^{-1} 4-methyl-n-valeric (i-caproic) acid as an internal standard. The tubes were sealed and the contents mixed by inverting them 10 times. They were then left for three minutes before centrifugation at 4500 rpm for a few seconds. Two liquid layers were formed within the tube, the upper being diethyl ether containing VFAs. Later samples and standards were not centrifuged due to problems with the rotor of the centrifuge but there was no apparent difference in calibration of the GC or running of samples. The top layer of liquid was removed with a pipette and placed in a 12x32 mm screw-cap vial (Alltech, UK) with open top screw caps lined with red TFE/silicone liners (Alltech, UK). This seal was necessary for pressurised filling of the injection system. The standards and samples were run as recommended by the manufacturers. The calibration was verified using a standard each time samples were run, and recalibration carried out as necessary. Volatile fatty acids can adsorb to the column and therefore following injection of samples at least one wash containing 10% formic acid in diethyl ether was injected to clean the column. The standard error for this system has been cited as less than 2% for each VFA

(Peck *et al.*, 1986). The VFAs measured in the STE were due to the addition of acetic acid to the STE. The mean TVFA concentration was calculated subsequent to days 20-30, Experiment 1 when acetic acid was omitted from the STE due to an error.

2.5.3 Gas Chromatography.

Gas chromatography was carried out to determine the percentage of carbon dioxide and methane in the biogas produced by the anaerobic digesters. A Varian Star 3400 CX GC with a 2 ft steel column packed with Porapak N80-100 (SUPELCO Inc. USA) and thermal conductivity (TC) detector was used. This detector acts as a circuit which is disrupted when solute molecules elute, thus enabling detection of the components (Fowlis, 1995). The Varian Star was linked to a workstation. The system set-up was that described by Varian Associates Inc. (1993) and the method was that recommended by the manufacturers (Carrier gas: Helium; Flow rate: 10-80 ml min^{-1}). The column, injector and detector were set at temperatures of 60°C, 110°C and 200°C respectively.

A single point calibration was carried out using a standard gas (BOC Gases, Guildford, UK) with a carbon dioxide:methane ratio of 40:60. If the calibration was satisfactory (i.e. within 5% of the correct concentration) gas samples were analysed. Gas samples and standards were taken using a syringe fitted with a 3-way valve. The syringe was flushed with 10 ml of standard gas to ensure that the sample taken was representative. The second aliquot was manually injected into the GC machine. The sample port for gas samples on the anaerobic reactors was located on the main gas line leading from the reactor prior to the Drexel bottle. The Varian Workstation calculated the percentage of methane and carbon dioxide in the sample from the peak area. The standard error in use of this equipment was less than 0.5% (Peck *et al.*, 1985). The biogas did not form 100% of the gas collected as the sampling procedure introduced a small quantity of air into the syringe.

2.6 Measurement of Oxygen Demand.

2.6.1 Chemical Oxygen Demand (COD).

The COD of samples was measured using a sealed tube method (HMSO, 1986). The detection range of this method was 9-400 mg l^{-1} COD, so concentrated samples required dilution with ultrapure water. In Experiment 1 samples were allowed to settle for approximately 1 hour prior to preparation, while in subsequent Experiments they were centrifuged at approximately 3000 rpm for 5 minutes. Equipment was kept solely for COD measurement to avoid contamination problems. This method was similar to that recommended by the American Public Health Association (1995) with the exceptions that 5 ml of 'Ficodox plus' COD reagent (Fisher, UK) was added prior to digestion rather than individual reagents, and samples were titrated against 0.0125 M FAS. Three replicates were prepared of blanks and samples. The FAS was standardised by titration in triplicate against a solution containing ferroin indicator, 2.5 ml of 0.0418 M (0.25 N) potassium dichromate, 25 ml of deionised water and 7.5 ml of concentrated H_2SO_4. Standard deviations of up to 5.6% have been cited for the closed reflux method (American Public Health Association, 1995) although HMSO (1986) cited standard deviations of up to 9.01% for industrial effluent samples. Guwy (1995) found precision between samples to be $\pm$ 100 mg COD l^{-1}. The mean standard deviation of replicates of the same samples of STE in days 6-123, Experiment 4 was found to be 4.85% (SD: 4.26; n: 78). The mean COD measurements for Experiments 1 and 2 were taken subsequent to days 20-30, Experiment 1, during which period acetic acid was omitted from the STE due to an error.

2.6.2 Biochemical Oxygen Demand (BOD).

The five day biochemical oxygen demand (BOD) was measured using the WTW OxiTop 1230T system (Burmarc Ltd., UK) with a method based on WTW Application Report Number BSB 296231 using 250 ml volumes. Samples were required to be within the range of 0-200 mg l^{-1} BOD for this method so different volumes of sample were added to the bottles to take account of this. The required sample volume was estimated from the

COD of the samples using the assumption that COD:BOD was 3. Dilution water was made up for the experiment as recommended by the Standing Committee of Analysts (1983). To each 500 ml of dilution water, the contents of one polyseed capsule were added. This ensured the presence of a high concentration of viable bacteria and provided consistency in seeding between tests. The dilution water was aerated and stirred for one hour using a Capex L2C compressor (BDH, UK).

Three replicates were prepared of blanks, containing dilution water only, and samples. A rubber quiver containing two sodium hydroxide pellets was placed in top of each 500 ml bottle. The lids were screwed on hand tight and the two buttons on the lids pressed simultaneously to start the sensor. The bottles were placed in an incubator at 20°C. A pressure reduction occurred within bottles containing biodegradable material as oxygen was consumed during respiration and the carbon dioxide produced was absorbed by the sodium hydroxide. A respirometric electronic pressure measurement was carried out every 24 hours until the 5 day period was complete. The final value recorded (M_5) was used to calculate the BOD using the equation recommended by the manufacturers as follows:

$$\text{BOD of sample} = \frac{F.M_5 \times (V_v + V_p)}{V_p} - BW$$

F-Multiplication Factor-5
M_5-Measured value of OxiTop on day 5
V_v-Volume of dilution water in the dilution
V_p-Volume of sample in the bottle
BW-Blank (value)

The value obtained for the blank was corrected to take account of the different amounts of dilution water in the sample bottles.

$$BW = \frac{F \times M_5 \times V_v}{V_p}$$

A colleague later implemented a change in the method. This involved adding a constant 50 ml of dilution water to each bottle. Sample was added as appropriate, and samples

and blanks were made up to 250 ml with aerated deionised water. This reduced the error associated with the addition of the different quantities of dilution water. V_v then became 50 ml and V_p became the diluted sample volume of 200 ml. The value obtained from the calculations above was then multiplied by $^{200}/_{v}$, where v was the ml of sample originally added to the bottle, to compensate for the dilution factor. The BOD for experiments containing 0.45 g l^{-1} dye, 2.9 g l^{-1} starch was carried out using this slight modification. All other BOD tests were carried out using the previous method. A standard deviation of 18.6% has been cited for the older method of BOD measurement (American Public Health Association, 1981) with more recent citations of 5.1-15.4% (American Public Health Association, 1995). Up to 20% variation has been cited for this test when carried out over longer periods using activated sludge rather than polyseed capsules (Conzelmann, 1996).

The BOD for each day could be calcuated from the daily readings taken in each bottle. A graph of the BOD for each replicate of a sample of simulated textile effluent vs day can be seen in Figure 2.5. It was seen that the BOD had peaked by day 3 showing that a 5 day period was adequate for the measurement.

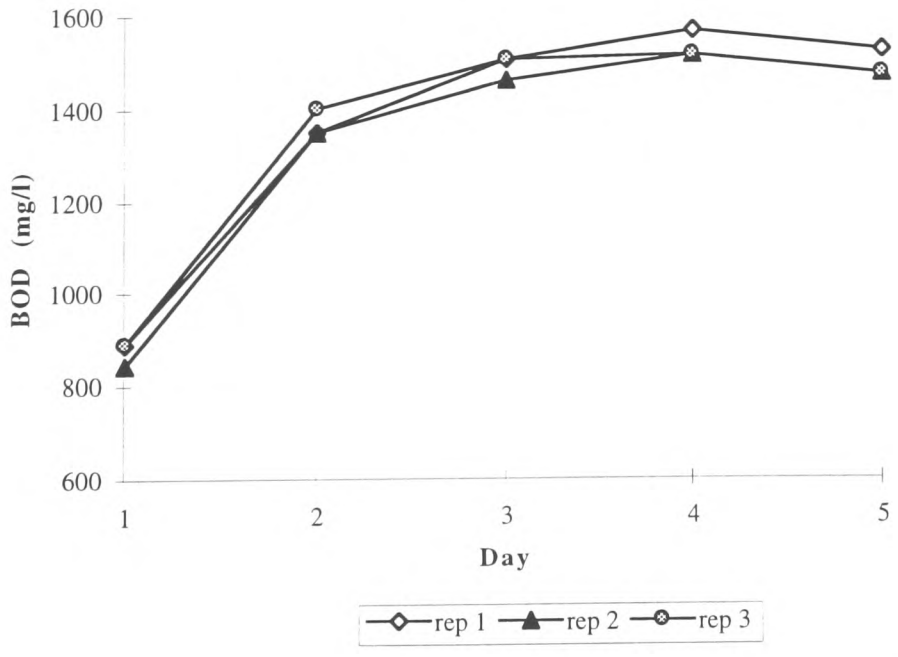


Figure 2.5 BOD Of Three Replicates Of STE (0.15 g l^{-1} Dye, 1.9 g l^{-1} Starch) For Each Day Of BOD Test.

Final effluent samples from Experiment 1 through to Expt 4.4 were not filtered prior to BOD measurement. It was subsequently found that the presence of aerobic biomass in the final effluent led to an over-estimate, by approximately 43%, of the BOD. This could have been due either to the biomass providing an additional source of carbon in this test, or to the addition of biomass providing a greater oxygen uptake in a five day period. Samples from Expt 4.1-4.4 were adjusted to take account of this. Samples from previous Experiments were not adjusted, as the precise cause of the error was unknown. Prior to Experiment 4 the sludge was returned only periodically and was therefore likely to have been less active. If the error was attributable to higher respiration rate, then the error would have been smaller in these previous Experiments. Final effluent samples from Expt 4.5 were filtered.

2.6.3 Total Organic Carbon (TOC) and Total Oxygen Demand (TOD).

Methods used for both these instruments were those recommended by the manufacturers. The TOC of samples was measured in triplicate on a Rosemount Dohrman DC-190 Total Organic Carbon analyzer (Sartec UK. Ltd., UK). The TOC measurement does not detect the contribution of compounds other than carbon (e.g. nitrogen) to COD or BOD. The TOC analyzer determined the inorganic carbon content of samples by measurement of the quantity of carbon dioxide liberated after contact with acid. The total carbon content was then determined by measuring the quantity of CO_2 liberated through combustion of the sample. The total organic carbon concentration was the difference between the two measurements. The CO_2 was measured by an infrared detector.

A colleague measured the TOD of samples on an Ionics Model 7800 E Total Oxygen Demand Analyser (Ionics, UK). Samples were run through a 185 μm filter prior to entering the machine in order to remove large solids. Samples were combusted in a high temperature combustion chamber. Oxygen which diffused into the carrier gas, in this case nitrogen, was measured before and after combustion by means of a high temperature

zirconia oxygen probe. The difference between the readings was the oxygen demand of the sample.

2.7 Solids Measurements.

Solids measurements made were Total Solids (TS), Total Suspended Solids (TSS), Mixed Liquor Suspended Solids (MLSS), Volatile Solids (VS) and Volatile Suspended Solids (VSS). Methods used were those recommended by the American Public Health Association (1995). The MLSS is the total suspended solids measurement performed on aerobic biomass. The VSS measurement was used to determine how much of the solids were bacterial, as inorganic solids are not volatile at 550 °C. Results obtained from TS and VS are higher than the corresponding TSS and VSS measurements as dissolved solids are included in the former measurements. The precision of TS measurements has been cited as $^{+}/_{-}$ 5% (American Public Health Association, 1981). Standard deviations of 0.76-33% have been found in samples containing 15-1707 mg l^{-1} TSS, increasing with decreasing sample size. A standard deviation of 6.5% has been found in samples with a mean VS of 170 mg l^{-1} (American Public Health Association, 1995). The variation differs with sample type and size and so can be difficult to predict. The mean standard deviation between replicates of the same sample for MLSS was found to be 5.4% (SD: 4.9; n: 77), and for VSS was 4.4% (SD: 3.5; n: 58) for samples taken from days 8-123, Experiment 4. The mean measured MLSS and VSS in this period was 2.34 g l^{-1} (SD: 1.54; n: 77) and 1.96 g l^{-1} respectively (SD: 1.96; n: 75).

2.8 Coagulation.

A range of coagulants was tested to determine whether simulated textile effluent could be coagulated efficiently as described in Table 2.3.

The Magnafloc coagulants were highly cationic organic liquids. DEC 50 was a synthetic polymer, and the Ökoflock K5600 was a cationic compound based on acrylamide and

Table 2.3 Coagulants And Dye And Starch Concentrations In The STE With Which They Were Used.

Coagulant	Supplier	+Conc.	STE Dye ($g\ l^{-1}$)	STE Starch ($g\ l^{-1}$)
Magnafloc 1597	Allied Colloids,	1:10	1.5	1.9
Magnafloc 1697	Bradford, UK.	1:10	1.5	1.9
#Magnafloc 1797		As supplied	1.5	1.9
*Magnafloc 919		0.05% (w/v)		
DEC 50	Aqua Ambiente,	As supplied	1.5	1.9
	Portugal		0.075	0.95
Ökoflock	Sepulchre,	0.1% (w/v)	1.5	1.9
	Brussels, Belgium		0.075	0.95
Iron sulphate	Fisher, UK	5 g l^{-1}	1.5	1.9
		10 g l^{-1}	0.075	1.9
Iron chloride		5 g l^{-1}	1.5	1.9
		10.8 g l^{-1}	0.075	1.9
Aluminium sulphate		5 g l^{-1}	1.5	1.9
Aluminium chloride		5 g l^{-1}	1.5	1.9

*Flocculant. #Baird Dyers (UK) reported better results with use of concentrate (J. Stonehewer, pers. comm.). +Conc. - concentration of coagulant in solutions added to the samples.

acrylic-acid aminoester copolymers. Two flocculants were examined: Magnafloc 919 and Zetag 78 FS40. The former was an ultra high weight anionic polyacrylamide. The latter was difficult to get into solution and therefore was not used.

Approximately 100 ml of STE were placed in a beaker and stirred at high speed for three minutes using a magnetic stirrer while coagulant was added. In the case of the commercial coagulants, 0.5 ml aliquots (i.e. 5 ml l^{-1}) were added either until coagulation was achieved or until 5 ml (i.e. 50 ml l^{-1}) had been added. The solutions of chemicals used with STE containing 1.5 g l^{-1} dye were added until approximately 50 ml had been added or coagulation had been achieved. A range of ferrous sulphate and ferric chloride concentrations were tested with STE containing 0.075 g l^{-1} dye. Samples were then stirred slowly for 10 minutes before being left to settle.

In order to test the effect of addition of flocculant on coagulated samples, a 0.05% solution of Magnafloc 919 was prepared in accordance with manufacturer's instructions.

Samples were coagulated with Magnafloc 1597, 1697 and 1797. After three minutes of stirring rapidly, 1 ml of flocculant was added and the speed was immediately reduced to a very slow speed. Samples were stirred for ten minutes and then left to settle.

2.9 Spectrophotometry.

2.9.1 Ultraviolet (UV) Spectrophotometry.

The spectra of samples were examined using an ATI Unicam UV1 spectrophotometer (ATI Unicam, UK) and a plastic cell with a 1 cm path length. Samples for true colour measurement were centrifuged at approximately 3000 rpm for 5 minutes prior to UV measurement. Samples were scanned in the visible spectrum using a 2 nm bandwidth. The true or apparent colour of samples was determined by measurement of the average optical density at 436, 525 and 620 nm in centrifuged and uncentrifuged samples respectively in a manner similar to that described elsewhere (British Standards Institution, 1995). This was achieved by scanning the spectra of samples and reading the absorbance at the required wavelengths. Initially apparent colour measurements were carried out in addition to true colour measurements. However, subsequent to Experiment 1 only true measurements were taken, as assessment of colour removal was based on this parameter. The results were expressed as absorbance (abs) units. At an absorbance of 1 the manufacturers reported an error of 0.005, or 0.5%.

2.9.2 American Dye Manufacturers Institute Units (ADMI).

The ADMI method for assessing colour is that stipulated by the American Dye Manufacturers Institute and the measurements are referred to as ADMI colour values. A stock standard of 500 ADMI units was prepared as recommended by the American Public Health Association, (1995). Standards of 25, 50, 100, 150, 200 and 250 ADMI units were then prepared by dilution of the stock with deionised water. Only dye solutions were measured and therefore the recommended filtration step was omitted. Samples were measured at pH 7.6. The percentage transmittance of samples and

standards at 30 sets of wavelengths (American Public Health Association, 1995) were measured on a spectrophotometer with a 1 cm cell path (Section 2.9.1) with the aid of a computer program called ASDS (ATI Unicam, UK). A 100% line was also measured with both cells filled with deionised water. The tristimulus values of water, samples and standards were calculated as described by the American Public Health Association (1995). These values were converted to the corresponding Munsell values by the use of published tables (McLaren, 1970). An intermediate value, DE, was calculated from the Munsell values as described by Allen *et al.* (1973) and the American Public Health Association (1995). The ADMI values of the standards were plotted against their calculated DE values (Figure 2.6). The ADMI values of samples analysed were then read off this graph from the corresponding DE values.

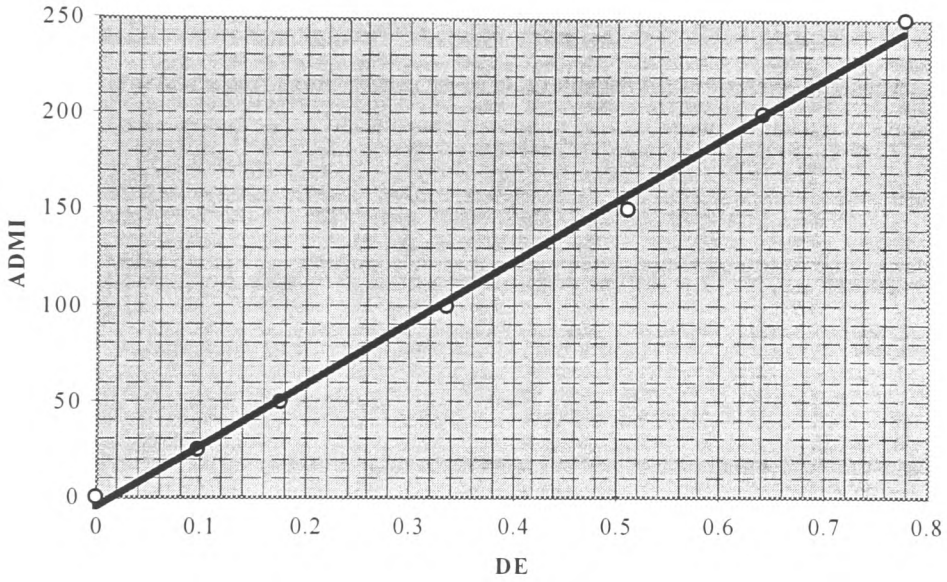


Figure 2.6 Plot Of ADMI Against Calculated DE For Standards Ranging From 0-250 ADMI Units.

2.9.3 Infrared (IR) Spectrometry.

Infrared spectrometry was carried out to determine whether the presence of aromatic amines could be detected by this method. The energy of most molecular vibrations can be detected in the infrared region of the electromagnetic spectrum, between 636-4000 cm^{-1}. The infrared beam was split in two, one being the reference beam and the other passing through the sample. If the frequency of vibration of a molecule in the sample fell within

the range of wavelengths at which IR light was emitted by the instrument, the molecule absorbed energy of this frequency. Comparison of the intensity of the two beams therefore enabled determination of where in the spectrum absorption occurred. In complex molecules much vibration occurs. Some of this is associated with the vibrations of individual bonds or groups and some with vibrations of the entire molecule. The IR spectrum plots percentage transmittance against the reciprocal of the wavelength and the downward peaks show where absorption occurs. The many different types of vibration, including stretching, bending and rocking, mean that some molecules can be detected in several regions of the spectrum.

Samples were initially dried in an oven at 105°C to remove all the water. They were then prepared as described by Williams and Fleming (1995) using the KBr disc method, which produced interference-free spectra. Samples were ground with approximately 40 times their volume of KBr. A small amount of the resulting mixture was placed into a 13 mm die which acted as a mould (Specac, Sidcup, UK). The mould was then pressurised using ten tons of pressure on a 25 ton Ring Press 00-25 (Research and Industrial Instruments Co., UK) to produce a disc. A percentage transmittance of approximately 50% was required. Discs that were too thin broke easily and thus could not be used, and discs that were too thick did not let through sufficient light for the spectrum to be plotted. Prepared discs were put in a sample holder and scanned using a Perkin Elmer 881 Infrared Spectrophotometer (UK).

2.10 Biodegradability and Toxicity Tests.

2.10.1 Anaerobic Biodegradability Tests.

The anaerobic biodegradability of samples with known composition was determined in accordance with the method recommended by the Standing Committee of Analysts (1988). As the composition of the samples was known, the carbon content was calculated from the formulae of the sample constituents. Glass amber bottles (125 ml) were used with open top screw caps sealed with heavy duty 22/400 teflon/silicone liners

(Alltech Associates, UK). The dilution medium, which consisted primarily of nutrients and trace elements, was not autoclaved. The medium was seeded with anaerobic sludge from Pen-y-Bont sewage treatment plant to give a final concentration of 2-3 g dry solids l^{-1}. All samples were prepared in triplicate, including distilled water blanks and a control containing 14 µl of 90% ethanol. The gas produced by each sample was measured with glass syringes while holding the bottles underwater to prevent escape of gas. Further measurement of gas production was performed at a later stage, if required, to measure long-term degradation. The blanks provided a measurement of the gas production of samples in the absence of a carbon source. The net gas production was calculated by subtracting the gas produced by the blank from that produced by each of the sample bottles. It gave a measurement of the sample's potential to be anaerobically degraded under methanogenic conditions. The control was used to determine whether or not the sludge was sufficiently active for this test. The calculations were carried out as described by the Standing Committee of Analysts (1988).

2.10.2 Respiration Inhibition.

Respiration inhibition is based on a measurement of the utilisation of oxygen by aerobic bacteria, assuming that the rate of oxygen uptake is proportional to substrate utilisation. Samples were sent away to Alcontrol Laboratories (UK) for this test as equipment was not available in the laboratory for this measurement. The method employed was developed by Alcontrol and accredited by the UK Accreditation Service (UKAS) (Alcontrol Laboratories, 1996).

Unacclimatised activated sludge was used for the test together with an Electrolytic respirometer (Meritox 20®). The sample was mixed with OECD synthetic sewage (OECD, 1981), to provide nutrients and food, and activated sludge containing bacteria, protozoa and other organisms. The mixture was incubated for three hours and the respiration rate calculated from the oxygen consumed between hours 2 and 3. If respiration of the sample was reduced compared to a control this indicated that the sample was toxic to at least some of the organisms in the sludge. The respiration

inhibition value indicated the percentage of toxicity by comparison to a control at the final dilution, e.g. n% toxicity x10 dilution. The dilution factor arose from the addition of the activated sludge and synthetic sewage. Respiration inhibition exceeding 10% was considered to be significant.

2.11 Other Gas Measurements.

2.11.1 Hydrogen Sulphide and On-line Carbon Dioxide Concentration.

Approximately 300 ml of biogas were collected in a gas bag (Alltech Associates, Lancs., UK). Hydrogen sulphide Kitagawa precision gas detector tubes (0.1-4.0%) were then used (Alltech Associates, Lancs., UK) in conjunction with a Matheson Toxic Gas Detector Model 8014KA (Alltech Associates, Lancs., UK) as recommended by the manufacturer in order to determine the quantity of H_2S present.

A colleague monitored the percentage of carbon dioxide in the biogas on-line as described by Guwy *et al.* (1997) using an ADC monitor type SBG100-002-15290 (ADC Ltd. Hodderson, UK). Gas flowing through this monitor was recycled at 19 ml min^{-1} to the gas space of the UASB. This measured CO_2 with an infrared detector.

2.11.2 Gas Production.

Gas from all anaerobic reactors was sent to a gas meter as described by Guwy *et al.* (1995). This was designed to measure low rates of flow (<5 cm^3 min^{-1}) and operate continuously. It was not affected by irregular gas production, and did not cause sudden large changes in pressure, which could have displaced liquid from the reactor.

CHAPTER THREE - GENERATION, CHARACTERISATION AND POTENTIAL PRE-TREATMENT OF SIMULATED TEXTILE EFFLUENT.

3.1 Use of Simulated Textile Effluents.

This chapter discusses the generation of a simulated textile effluent (STE). A mixed cotton processing effluent was simulated in order that the waste would contain starch to provide the carbon required for reduction of the azo bonds in anaerobic systems (Section 1.5.2.1.1). Due to the variability in textile wastewater composition, no artificial waste can be truly representative, either of a particular type of waste or even of a particular factory (O'Neill *et al.*, 1999c). However, for the purposes of this research it was attempted to generate an STE that broadly reflected the characteristics of real textile effluent, but containing only the principal compounds normally found in textile effluent and a dye of known structure. The advantages of using STE are given in Section 1.5.

Any solution containing one or more dyes can be considered to be a simulated textile effluent. Therefore published work on anaerobic decolourisation of dyes (Table 1.6) can also be considered to review simulated textile effluents. A number of researchers have used artificial textile wastes or other solutions containing textile dyes in the investigation of treatment technologies. Some of these are listed in Table 3.1. With a few exceptions, concentrations of dye used in STEs (Table 3.1) mainly varied from 0.01 g l^{-1} to 0.5 g l^{-1}, within the broad range of 0.01-0.8 g l^{-1} in real effluents cited in Section 1.4.1. The concentration of 7 g l^{-1} dye used by Koprivanac *et al.* (1992, 1993; Table 3.1) greatly exceeded the normal range of concentrations. Although the authors said it was representative of waste streams in the reactive dye industry it may be representative only of Croatia, a particular factory, or of very concentrated effluent (O'Neill *et al.*, 1999a). The 2.5 g l^{-1} dye used by Wilcock *et al.* (1992) was also comparatively high. The variation in STE dye concentration can be attributed to a number of factors. These include the difficulty in finding figures relating to actual concentrations of dyes in real wastes, as most authors discuss colour in terms of absorbance or ADMI values; the

variation of dye concentration in effluent with dye type due to different exhaustion rates; and the variability in effluent produced by the textile industry (O'Neill *et al.*, 1999a).

Table 3.1 Dye Concentrations Used By Some Authors In Dye Solutions And Simulated Textile Effluents Used For Investigation Of Treatment Methods.

Author	Year	Concentration ($g\ l^{-1}$)	Type
Basibuyuk and Forster	1997a	0.025, 0.2	Acid, Basic
Basibuyuk and Forster	1997b	0.025, 0.05	Basic
Carliell *et al.*	1994b; 1995	0.1; 0.1-0.2	Reactive
Carrière *et al.*	1993	0.1, 0.5	Disazo acid
Chang *et al.*	1996	0.01	Basic
Jia *et al.*	1999	0.05	Reactive, acid, direct, sulphur, vat & others
Kace and Linford	1975	0.1	Disperse
Kang and Chang	1997	0.02	Reactive
Kirby *et al.*	1995	0.5	Reactive, diazo, azo, disperse, phthalocyanine
Koprivanac *et al.*	1992, 1993	7	Reactive
Li and Zhang	1996	0.1	Reactive, Disperse, Sulphur, Direct, Vat
Márquez and Costa	1996	0.02	Acid
Panswad & Wongchaisuwan	1986	0.3	Reactive
Perkowski *et al.*	1996	0.1	Acid, direct
Wilcock *et al.*	1992	2.5	Disperse
Yeh and Thomas	1995b	0.025-0.2	Disperse
Zissi and Lyberatos	1996	0.010	Disperse

3.2 Components of Simulated Textile Effluent (STE).

The composition of the STE used in this project was based on some of the principal components of real effluent and their contribution to effluent COD and BOD (Section 1.4). Components such as surfactants, oil and grease contained in real wastes were not included in the STE used here in order to prevent the reactions occurring within the treatment system from becoming too complex. Therefore this STE contained only the most common pollutants: dye, size and salt. Acetic acid was also added.

3.2.1 Dye.

Reactive dyes do not adhere to glass or perspex, making them ideal for laboratory use. Just one dye, PROCION Red H-E7B (C.I. Reactive Red 141; BASF, Manchester, UK; Figure 3.1) was selected for addition to the STE. This was in order to keep the STE as simple as possible. Information obtained from the name of the dye is as follows: PROCION is the BASF trade name for reactive cotton dyes; red is the main colour of the dye; H-E indicates that it is used for hot dyeing and is an exhaust dye; and 7B indicates that it is a blueish red, or magenta shade (Kirk-Othmer, 1993). The product is described by the manufacturers as 'a preparation containing azo bis-monochlorotriazine reactive dye.' It has a pH of 9.1 and a solubility of 150 g l^{-1} at 20°C. The potential anaerobic breakdown products of this compound were described by Carliell *et al.* (1995) and are also illustrated in Figure 3.1. However, according to the literature (Section 1.5.2.1.1), only product (I) is likely to be formed through anaerobic digestion of this dye.

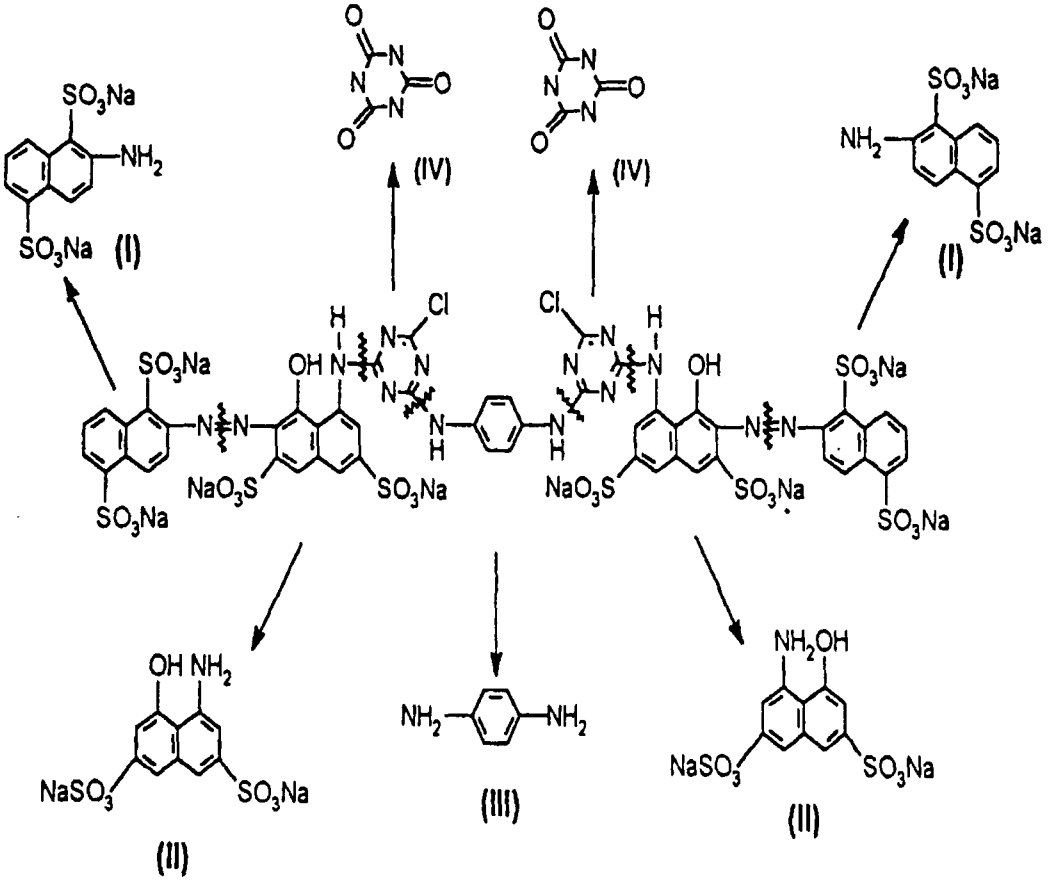


Figure 3.1 The Structure And Potential Anaerobic Degradation Products Of PROCION Red H-E7B (After Carliell *et al.*, 1995).

Reactive dyes as supplied normally contain 25-60% colour and PROCION Red H-E7B is no exception, containing 45% colour, 35% salt, 15% of a sodium lignosulphonate as a diluent, and 5% moisture. Small quantities of hydrocarbon dedusting agent are typically present. Small amounts of other colours, in this case PROCION Yellow H-E4R and PROCION Blue H-EGN, may be added to the dye to obtain the correct shade. Neither colour should be present at concentrations exceeding 2% (J. Easton, BASF, Manchester, UK, pers. comm.). PROCION Red H-E7B has low toxicity, with a 36 hour LC_{50} to rainbow trout exceeding 100 mg l^{-1}, similar to the majority of dyes (Section 1.4.2). It is said to be unlikely to inhibit aerobic bacteria and the manufacturers reported no evidence of inhibition to anaerobic treatment at 2.5% dry solids, i.e. 25 g l^{-1} (BASF, Manchester, UK). However, Carliell *et al.* (1995) reported that anaerobic biodegradability tests showed this dye to be inhibitory to unacclimated anaerobic micro-organisms at concentrations in excess of 100 mg l^{-1}, possibly due to its degradation products.

The dye manufacturers reported colour removal of <10% in activated sludge tests and 50-100% in anaerobic sludge digestion tests. Dyes tend to have low BOD values and high COD:BOD ratios (Goronszy and Tomas, 1992). Ratios of 2-3 indicate good potential biodegradability, but ratios of 5 or greater indicate poor biodegradability (British Textile Technology Group, 1996). It is rare for dyes to have a significant BOD that is not attributable to one of the diluents contained in the powder as supplied by manufacturers (Hitz *et al.*, 1978). PROCION Red H-E7B has a COD of approximately 600 mg g^{-1} and a BOD of <50 mg g^{-1} (BASF, Manchester, UK). Therefore the COD:BOD is 12, which means this dye, like most dyes, is not very biodegradable.

3.2.2 Size.

Approximately 75% of sizes used in the textile industry are starch-based (Weber and Ströhle, 1997). Corn, maize and potato starches are most commonly used (Allied Colloids, UK, pers. comm.). Tissalys 150 (Roquette UK, Tunbridge Wells, UK) is a commercial potato starch widely used in the cotton industry. It is a white powder with 19% maximum weight loss on drying, a value typical of potato starches. This loss is presumably attributable to the 17% moisture content. It is a starch acetate ester of low

viscosity, manufactured by acetylating purified potato starch under carefully controlled pH, temperature and time conditions. The acetyl value is a minimum of 1.7%, which means that the amount of esterification is very low and only a small number of hydroxyl groups are affected. It is used for sizing natural yarns, natural and synthetic spun yarns, and blends. It is used alone for sizing cotton, but may be mixed with other compounds for sizing synthetic blends. A 20% suspension in water has a pH of 5-6. Starch can act as a co-substrate for anaerobic dye decolourisation (Section 1.5.2.1.1).

3.2.3 STE Composition.

The STE could be made up in the concentration required to be fed to the reactors, or made up as a concentrate and diluted with water (Section 2.1). Table 3.2a shows the composition of the STE, as it entered the anaeobic stage, used in Experiments 1 and 2. Non-analytical grade chemicals were used.

Table 3.2a STE Composition (When Diluted) Used In Experiments 1 And 2.

Component	Concentration ($g\ l^{-1}$)
Hydrolysed Starch (Tissalys 150)	1.9
Hydrolysed Dye (PROCION Red H-E7B)	1.5
Acetic acid	0.53
NaCl	1.5
NH_4Cl	0.23
$(NH_4)_2SO_4$	0.28
$Na_3PO_4.12H_20$	0.123
$Na_2HPO_4.12H_20$	0.096
Trace element solution	1 ml

Initially the pH of the STE was adjusted from approximately 9.4 to 7.5 using 1 M HCl. However, microbial action affects the pH of feed in anaerobic systems, which means that it is often not necessary, and sometimes detrimental, to pH adjust the feed (Speece, 1996). Therefore from day 23, Experiment 1 pH adjustment was not made. Initially 3.5 g salt l^{-1} was used but this was reduced to 1.5 g l^{-1} on day 23, Experiment 1, in order to reduce the potential for sodium toxicity. A trace element solution was added to the

STE (Table 3.2b). It was a composition used in the Microbiology laboratory in the University of Glamorgan, with the addition of nickel and use of $MnCl_2$ in place of $MnSO_4.4H_2O$.

Table 3.2b Composition Of Trace Element Solution.

Trace Element Compounds	Concentration (g l^{-1})
H_2SO_4 (concentrated)	4 drops
EDTA.Na	2.50
$FeSO_4.7H_2O$	5.0
$ZnSO_4.7H_2O$.	0.011
$MnCl_2.4H_2O$	0.1
$CuSO_4.5H_2O$	0.392
$Co(NO_3)_2.6H_2O$	0.248
$NaB_4O_7.10H_2O$	0.177
$NiCl_2.6H_2O$	0.025

A total of 18 mg P l^{-1} was added to the STE with equal contributions from tri-sodium orthophosphate (hydrated) ($Na_3PO_4.12H_20$) and di-sodium hydrogen orthophosphate dodecahydrate ($NaHPO_4.12H_20$). A concentration of 120 mg N l^{-1} was added with equal contributions from ammonium chloride (NH_4Cl) and ammonium sulphate ($(NH_4)_2SO_4$). The projected COD of this STE composition was 3150 mg l^{-1}, based on the COD of the dye as specified (600 mg g^{-1}), the calculated theoretical COD of acetic acid (1067 mg g^{-1}), and the calculated COD of the starch, taking into account the 17% moisture content (886 mg g^{-1}). This gave a projected COD:N:P of 175:6.7:1, assuming no N was available from the dye. When this was compared with the nutrient requirements cited in Section 1.5.2.1.2 it was seen that an excess of nutrients were present.

3.3 Tests Carried Out On STE.

The bicarbonate alkalinity of the STE was measured to determine how much buffering capacity was present. Ion exchange HPLC of STE samples containing 0.15 g l^{-1} dye together with both 1.9 and 0.95 g l^{-1} starch (days 67 and 74 respectively, Experiment 3) was carried out to determine whether the detected concentrations of nutrients were

similar to those estimated. The COD, BOD, TOC, TOD, TSS and volatile fatty acid concentration in the STE were also measured. The spectra of the STE and hydrolysed dye were examined in order to see where peaks arose due to the presence of the dye, and the true and apparent colour were determined. The ADMI of a 0.075 g l^{-1} solution of PROCION Red H-E7B was examined. This dye concentration was selected to obtain good transmittance values. These results were used to determine whether the STE was comparable with real effluent.

Coagulation of STE with a range of coagulants was investigated. Anaerobic biodegradability tests were carried out to discover whether the components of the STE were biodegradable by anaerobic means and hence whether anaerobic digestion was a suitable method of treatment for this STE. The samples tested were as follows: STE alone; STE without dye; STE without starch; hydrolysed starch alone; hydrolysed dye alone; and STE with 1 ml l^{-1} Magnafloc 1797. STE containing 1.5 g l^{-1} dye and 1.9 g l^{-1} starch (Table 3.2a) was used in these tests. Results were analysed using a statistical program called Data Desk® 4.1.

3.4 Results and Discussion.

The natural bicarbonate alkalinity of the STE was 80 mg $CaCO_3$ l^{-1} (SD: 6; n: 4) indicating little natural buffering capacity was present. Therefore sodium bicarbonate ($NaHCO_3$) was added to the water used to dilute the STE (Section 2.1), giving a final concentration of 1.5 g l^{-1} $NaHCO_3$ in the feed entering the reactor. This enabled the STE to be treated anaerobically without risk to the stability of the digesters.

3.4.1 Toxicity.

The contribution of sulphate from nutrients was 0.2 g l^{-1} STE. The sulphur from the sulphonic acid groups on the dye corresponded to a maximum of 0.10 g sulphur l^{-1} STE at 1.5 g l^{-1} dye. This corresponded to 0.3 g sulphate l^{-1} STE, giving a total of 0.5 g sulphate l^{-1} STE. This was below the 2-4 g l^{-1} sulphate cited as being inhibitory to anaerobic

processes (Section 1.5.2.1). Additionally the sulphonic acid groups were unlikely to be converted to sulphate. Therefore sulphate toxicity was unlikely to present a problem.

Sodium in the STE arose from the addition of NaCl, sodium bicarbonate, nutrients, hydrolysed starch, and the dye powder. The sodium additions from NaOH used in dye hydrolysis and from sodium lignosulphonate in the dye powder were excluded from this calculation. The highest total sodium concentration that could be present in the STE was 2.46 g l^{-1}, assuming 3.8 g l^{-1} starch; 1.5 g l^{-1} salt and dye; and 2.5 g l^{-1} sodium bicarbonate, used only during periods of reactor instability. This was below the concentrations cited as causing toxicity (Section 1.5.2.1) and therefore toxicity was unlikely to be experienced in this system. In practice the highest concentrations of salt, starch, dye and sodium bicarbonate were not used concurrently, further reducing the likelihood of toxicity.

3.4.2 Biodegradability Tests.

The carbon content of the samples in each bottle, the net gas production and the theoretical gas production for each sample can be seen in Table 3.3.

Table 3.3 Carbon Content In Each Bottle (mg/Bottle), Net And Theoretical Gas Production (ml) And The Percentage Degradation For Each Sample After 11 Days.

Sample	mg of C in bottles	Net GP (ml)			Theor. GP (ml)	% of theoretical degradation		
		mean	SD	n		mean	SD	n
Dye alone	2.4	-1	(0.3)	3	3.2	-31	(8)	3
Starch alone	5.07	7.14	(1.4)	3	6.9	103	(21)	3
STE without dye	6.48	10.9	(0.9)	3	8.9	123	(9.9)	3
STE without starch	4.52	1.3	(0.6)	3	6.0	22	(9.5)	3
STE	6.06	9.4	(0.8)	3	8.0	117	(10)	3
STE and coagulant*	6.06	9.0	(1.8)	3	8.0	112	(22)	3
Ethanol	5.3	9.3	(1.4)	3	9.0	103	(16)	3

mean (SD) n. A mean 4.5 ml of gas was produced by the blanks (SD = 0.25 ml).
GP - Gas Production. Theor. - Theoretical
coagulant - 1 ml l^{-1} Magnafloc 1797

Approximately 5 mg carbon was added to each bottle, with the exception of the dye alone. In that case the addition was based on the presumption that the dye powder was

100% colour, and the value recalculated at a later date when the manufacturers provided data showing that only 45% colour was present (Section 3.2.1).

According to the Standing Committee of Analysts (1988) the assessment of degradability of samples is as follows:

Complete degradation	≥ 80% ThGP
Not degraded	<30% ThGP
Inhibitory	Negative cumulative net gas production

On the basis of these criteria, the ethanol control was fully degraded proving that the sludge was suitably active for this test. The dye alone was not degraded and the gas production was lower than that achieved in the blanks, indicating that it may be inhibitory. The starch alone, STE and STE without dye were fully biodegradable but the STE without starch was not degraded (O'Neill *et al.*, 1999c). Some samples produced a gas volume in excess of the theoretical, possibly due to the presence of the additional nutrients in the STE. The STE containing Magnafloc 1797 exhibited slightly less gas production than the STE alone, but was still categorised as fully degradable. However, the concentrations tested here were lower than those used in the coagulation tests. Therefore further tests would have to be carried out to see if higher concentrations were fully degradable. Difficulty was experienced subsequently in obtaining anaerobic sludge that was sufficiently active for the test to be repeated.

Analysis of Variance (ANOVA) detected significant difference between the percentage theoretical gas production achieved by different samples (99.9% Confidence Interval (CI)). There was no significant difference between replicates for percentage of theoretical gas production achieved (probability (p): 0.3737). This showed that the test was meaningful. T-tests were carried out to determine whether there were any differences between the mean percentage of the theoretical gas production achieved by each sample. Statistically significant differences were found between the sample containing dye only and all other samples; and STE without starch and all other samples (99-99.9% CI). Therefore there were significant differences between samples that contained starch and

those that did not. The significant difference between the sample containing dye only and STE without starch was attributable to the presence of acetic acid and nutrients in the latter. There was no significant difference between samples of starch only, STE without dye and STE (p: 0.1952-0.4617), showing that samples containing starch did not differ significantly from each other. There was no significant difference between STE and STE with coagulant (p: 0.7624), indicating that Magnafloc 1797 could be added to the STE at a concentration of 1 ml l^{-1} without affecting anaerobic biodegradability.

3.4.3 Analysis Of STE.

Results obtained from analysis of STE used in Experiments 1 and 2 can be seen in Table 3.4.

Table 3.4 Characterisation Of STE used in Experiments 1 and 2 (With Added Sodium Bicarbonate) And Reported Range Of Parameters (Laing, 1991).

Analysis	Mean	SD	n	Reported Range
BA (mg l^{-1} $CaCO_3$)	1410	(305)	95	
pH	9.4	(0.7)	87	4-12
COD (mg l^{-1})	3473	(679)	118#	500-5000
BOD (mg l^{-1})	1127	(288)	2#	200-3000
COD:BOD	3.1:1			2.5:1-5:1
TOC (d201) (mg l^{-1})	2099		1#	
TOD (d208) (mg l^{-1})	3450		1#	
TVFA (mg l^{-1})	575	(176)	104	
Apparent colour (abs units)	5.65	(0.16)	2	
True colour (abs units	5.62	(0.12)	2	
TSS (mg l^{-1})	260	(40)	1#	50-500

each n is the mean of three replicates

The COD:BOD ratio of the STE was 3.1:1, indicating that the STE was biodegradable. The measured COD (Table 3.4) of the STE in Table 3.2a was similar to the projected COD (Section 3.2.3) when the standard deviation was taken into account. The TOC was lower than the COD, as expected. The TOD results were similar to the mean COD readings. The BA of the STE with added sodium bicarbonate was in excess of the

minimum 1000 mg l^{-1} cited (Section 1.5.2.1) as necessary for buffering anaerobic systems. Some acidification occurred naturally when no acetic acid was added to the STE (Section 2.5.2) to give a TVFA concentration of 92 mg l^{-1} (O'Neill *et al.*, 1999c).

Nitrogen in the STE was principally detected as ammonium, with a mean detected concentration of 126 mg N l^{-1}. Phosphorus was principally detected as phosphate with a mean concentration of 25 mg P l^{-1}. These results showed that all the nutrients added to the STE were detectable by means of HPLC. This gave a COD:N:P of 139:5:1 which complied with the recommended COD:N:P for anaerobic systems of <350:5:1. The BOD:N:P of 45:5:1 showed that nutrient concentrations were in excess of the 100:5:1 recommended for biological treatment systems in general (Section 1.5.2.1.2). The potential anaerobic degradation products of the dye (Carliell *et al.*, 1995) indicated that this dye, unlike some others (Section 1.5.2.1.1) was unlikely to give rise to readily available nitrogen or carbon in anaerobic systems. However, if the degradation products were broken down aerobically, they would provide a source of carbon and nitrogen for that stage (Section 1.5.2.1.1).

A sample of STE without dye was found not to have any significant peaks in the visible range. Therefore all peaks in the STE were attributable to the presence of dye. The peak associated with colour in this dye was located between 440 and 600 nm. There was no difference between the spectrum of hydrolysed and unhydrolysed dye samples at the same pH as hydrolysis only affects the reactive group. The spectrum of a 20-fold dilution of the STE can be seen in Figure 3.2.

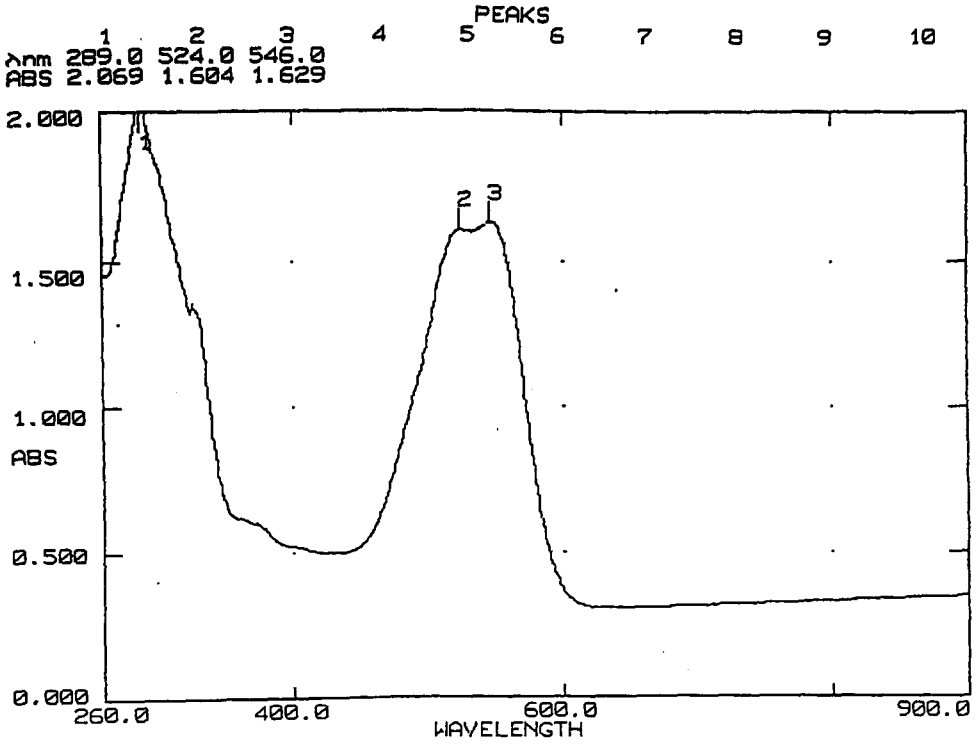


Figure 3.2 Absorption Spectrum Of A x20 Dilution Of STE (pH 10.2: Day 27, Experiment 1).

3.4.4 Comparison of STE with Real Textile Effluents.

It was seen from Table 3.4 that the parameters of the STE were within the typical ranges for mixed wastes given by Laing (1991). It was similar to real effluents in terms of COD, BOD, COD:BOD, pH, and TSS. Therefore the STE can be considered in many ways to be representative of real textile effluents to such extent as this is possible. However, it was far less complex. The ADMI of PROCION Red H-E7B was 8320 units g^{-1} of dye. Hence an ADMI of approximately 1500 units, considered to be reasonable for simulated cotton processing effluents (O'Neill *et al.*, 1999a) would give a concentration of 0.18 g l^{-1} dye. This was within the range of concentrations typically found in real and simulated textile effluents (Section 3.1).

The absorbance of a 'typical dyehouse end-of-pipe general site effluent' was compared with that of PROCION Red H-E7B (Table 3.5). At 500-550 nm the absorbance of the red dye was similar to or in excess of that of the site effluent, but at the other wavelengths was lower. This gave rise to a lower mean absorbance than the site effluent.

Therefore STE containing this one dye did not imitate the spectrum of the real effluent. A range of dyes would be required in order to imitate the colour of real effluents between 400-700 nm (O'Neill *et al.*, 1999a). However, this would render the STE more complex.

Table 3.5 Absorbance Of Typical Site Effluent (After Hoyle, 1995) And PROCION (P.) Red H-E7B.

Wavelength (nm)	Typical Site Effluent	P. Red H-E7B (0.125 g l^{-1})
400	1.423	0.521
450	1.529	0.472
500	1.965	1.774
550	1.715	2.121
600	1.514	0.065
650	0.772	0.004
700	0.62	0.003
Mean	1.283	0.709

3.4.5 Coagulation.

Concentrations of polyelectrolytes used by others with textile wastes include 11 ml l^{-1} (Sapari, 1996), 26-60 mg l^{-1} (Kace and Linford, 1975) and 80 ml l^{-1} (Baird Dyers, pers. comm.). Koprivanac *et al.* (1992) added 5-7.5 ml l^{-1} of cationic flocculant to reactive dye solutions. Kace and Linford (1975) treated effluent containing 600 mg l^{-1} of water-insoluble sulphur dye with 2 mg l^{-1} polyacrylamide and obtained 0 to 100 mg l^{-1} dye in the treated effluent. Concentrations of 300-600 mg l^{-1} ferric sulphate ($Fe_2(SO_4)_3$) (Sharma, 1989; British Textile Technology Group, 1996) and 300-600 mg l^{-1} alum (British Textile Technology Group, 1996; Sapari, 1996) have been recommended as necessary to achieve colour removal.

Concentrations of ~3 g l^{-1} ferrous sulphate coagulated STE (0.075 g l^{-1} dye, 0.95 g l^{-1} starch) but the flocs did not settle (Figure 3.3a). Some additional ferrous sulphate was added to the beaker depicted in Figure 3.3a subsequent to the 10 minute settling period,

a b c d

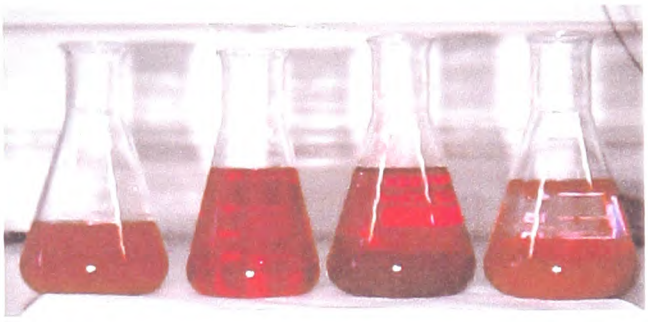
Figure 3.3 Coagulation Of STE Containing 0.95 g l^{-1} Starch, 0.075 g l^{-1} Dye, And 1 g l^{-1} PROCION Red H-E7B With Ferrous Sulphate.

a. STE coagulated at pH 11.5 with 3 g l^{-1} $FeSO_4$

b. STE coagulated at pH 4 with 5 g l^{-1} $FeSO_4$

c. PROCION Red H-E7B (1 g l^{-1}) coagulated at pH 11.5 with 10 g l^{-1} $FeSO_4$

d. STE coagulated at pH 11.5 with 8 g l^{-1} of $FeSO_4$.



a b c d

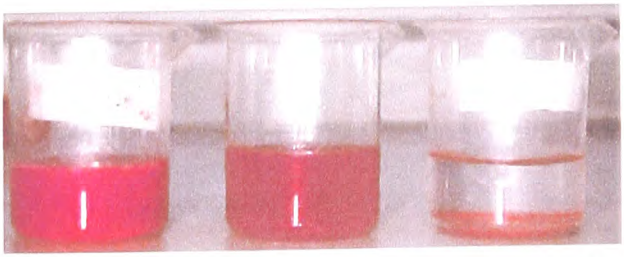
Figure 3.4 Coagulation Of STE Containing 0.95 g l^{-1} Starch, 0.075 g l^{-1} Dye, And 1 g l^{-1} PROCION Red H-E7B With Ferric Chloride.

a. STE treated at pH 11.5 with 5.4 g l^{-1} $FeCl_3$

b. STE treated at pH 4 with 10.8 g l^{-1} $FeCl_3$

c. Dye (1 g l^{-1}) treated at pH 11.5 with 17 g l^{-1} $FeCl_3$ (pH adjusted during test also)

d. STE treated at pH 11.5 with 16 g l^{-1} $FeCl_3$.



a (pH 4) b (pH 7) c (pH 11.4)

Figure 3.5 Coagulation Of STE Containing 0.95 g l^{-1} Starch, 0.075 g l^{-1} Dye With 10 ml l^{-1} DEC50.

but the flocs still did not settle. Concentrations of 8 g l^{-1} ferrous sulphate were successful for treatment of STE and settling was rapid (Figure 3.3d). However a large volume of sludge was produced which would generate high disposal costs in an industrial situation and therefore render use of this chemical impractical. It was found that high pH values were optimum, in accordance with Kang and Chang (1997) who found best colour removal at pH ≥10. Therefore pH adjustment of the wastewater to be treated might be required. Anaerobic treatment of either fraction of the coagulated effluent would introduce large quantities of sulphate into the system that could be detrimental (Section 1.5.2.1). Subsequent formation of H_2S would lead to problems of odour in addition to toxicity.

Ferric chloride and aluminium chloride did not coagulate STE containing 1.5 g l^{-1} dye, 1.9 g l^{-1} starch. However, iron chloride removed colour from STE with 0.075 g l^{-1} dye, 0.95 g l^{-1} starch (Figure 3.4). Again, the process was pH dependent and large quantities (~16 g l^{-1}) were required to achieve coagulation, which generated large quantities of sludge. The optimum pH was found to be 11.5. This differed from the pH 2.5 recommended by Koprivanac *et al.* (1993), who found 22-29 g l^{-1} of this chemical was required to coagulate solutions containing 7 g dye l^{-1}. The higher quantity of ferric chloride used by these authors was doubtless due to the higher dye concentration tested.

Ökoflock did not coagulate the STE at either 1.5 or 0.075 g l^{-1} dye. Magnafloc 1797 was the best of the Allied Colloids coagulants when used on STE containing 1.5 g l^{-1} dye, 1.9 g l^{-1} starch. However the coagulated particles did not settle upon standing and therefore a flocculant was required. When Magnafloc 919 was used as a flocculant, subsequent to coagulation with Magnafloc 1797, good settling was achieved after approximately two hours settling time. DEC 50 coagulated STE at both dye concentrations and good settling was eventually achieved (Figure 3.5). The settling time required was 3-4 hours at a dye concentration of 1.5 g l^{-1}, making use of this coagulant impractical on a small scale. However, at a concentration of 0.075 g l^{-1} dye a shorter settling time of about 45 minutes was required.

It was established, in line with other authors, that high concentrations of iron sulphate and iron chloride were required for coagulation with high associated sludge volumes, rendering their use impractical (Section 1.5.1). A colleague in IST (Portugal) carried out tests with coagulants and flocculants using STEs and real textile effluents. It was found that STEs showed poor coagulation characteristics with a range of products that coagulated real textile effluents efficiently. In order to coagulate STE prior to biological treatment it would be necessary to find a product that was not detrimental to biological processes, that ideally did not require pH adjustment, and that did not require excessive settling times, although this might be less important on an industrial scale. Any pH adjustment might require a second adjustment subsequent to coagulation in order not to exceed the limits of tolerance of biological treatment, thus increasing the concentrations of ions such as Na^+. Given the potential problems associated with addition of coagulants to STE and the length of settling times required in some cases, it was decided not to use coagulants prior to biological treatment.

3.5 Conclusions.

An STE was generated here which was less complex than true wastewaters and contained only one dye. It was similar to real effluents in terms of COD, BOD, COD:BOD, pH, and TSS. The principal difference between the STE and real effluents was in the spectrum. At typical ADMI values, i.e. 1500 ADMI units, the concentration of the dye used here was within the normal range of concentrations found in textile effluents. Therefore the STE can be considered to be representative of real textile effluent, to such extent as this is possible.

Nutrient concentrations were in excess of the minimum recommended for anaerobic systems, even at the highest STE CODs.

Sulphur and sodium toxicity were unlikely to arise in anaerobic treatment of the range of STE compositions tested here.

It was found that although dye alone was not degraded in anaerobic biodegradability tests, the STE was degraded. The COD:BOD of 3.1:1 also indicated that the STE was degradable. It was therefore concluded that anaerobic-aerobic treatment was suitable for this waste.

It was concluded that none of the coagulants tested were suitable for treatment of this STE prior to biological treatment due to problems of toxicity, pH adjustment, high sludge volumes produced by some coagulants and long settling times associated with others.

CHAPTER FOUR - ASSESSMENT OF TREATMENT OF SIMULATED TEXTILE EFFLUENT WITH AN ITD, UASB AND ACTIVATED SLUDGE TANK.

4.1 Introduction.

This Chapter discusses Experiment 1. This Experiment used an inclined tubular digester (ITD; Section 2.2.1) to examine the anaerobic degradation of STE containing 1.5 g l^{-1} dye and 1.9 g l^{-1} starch (Table 3.2a) at different hydraulic retention times. Applications of inclined tubular digesters on a laboratory scale include those of Buhlert *et al.* (1981), Hawkes *et al.* (1981), Chapman (1986), Floyd (1984) and Gorécki *et al.* (1993). However the information available on these digesters was limited and none was found on their use with textile waste. A 5 l UASB (Section 2.2.2.1) was used in addition to the ITD from day 150 in order to determine which would be the most suitable for anaerobic treatment of the STE. UASBs have been used in previous studies to treat dyes and textile effluent (Table 1.6; Zhu *et al.*, 1994; An *et al.* 1996; Donlon *et al.*, 1997; Razo-Flores *et al.*, 1997; Kuai *et al.*, 1998). An aerobic stage was placed subsequent to the anaerobic treatment from day 183 to further treat the effluent from both anaerobic digesters and to determine its biodegradability.

The performance of the ITD and UASB was assessed to determine which was the most suitable for treatment of STE (Table 3.2a) in terms of loading rates, colour removal and COD removal. The role of the aerobic stage in treatment of this effluent was also assessed. The starch:dye ratio in this Experiment was 1.27, with the starch and dye contributing 53 and 29% respectively to the projected COD. Simulated textile effluent and ITD effluent samples were analysed using Infrared spectrometry to determine whether it was possible to tell by this method whether amines were produced during anaerobic treatment of STE. Thus an attempt was made to determine whether colour removal occurred by means of degradation or adsorption.

4.2 Methods.

4.2.1 Reactors and Experimental Design.

The STE used was that described and characterised in Tables 3.2a and 3.4 respectively. Diluted STE was fed to the reactors until day 184, from which point a x2 concentrate was used. The ITD was seeded initially with five litres of anaerobic sludge from a Yorkshire Water municipal wastewater treatment plant that treated effluent from textile manufacturing plants. Therefore it may have already contained an acclimated population of bacteria and hence it was hoped that the acclimatisation period required for the bacteria to adapt to the STE would be short. Prior to Experiment 1, one litre of STE was added to the ITD. A further litre was added every day until the reactor was full. The ITD was fed intermittently for a 37 day start-up followed by 7 days of continuous feeding prior to commencing this series of measurements on day 1. Due to the long start-up period, the ITD can be considered to be in steady state from day 1 of this Experiment. It was operated continuously from days 1 to 77. The volumetric loading rates of the ITD were based on the 8.5 l active volume (Section 2.2.1). A volumetric loading rate (B_v) of 0.99 g COD l^{-1} d^{-1} (3.5 d HRT) was used from days 1-28. The B_v was increased to 1.24 g COD l^{-1} d^{-1} (2.8 d HRT) on day 28. The ITD was given a step loading on day 69 by increasing the B_v to 2.48 g COD l^{-1} d^{-1} (1.4 d HRT). There was a 51 day hiatus in measurements from day 77 when the ITD was fed at 2.8 d HRT for five days and then heated for 2 days unfed, followed by a period of 44 days at room temperature, unfed. During this period the ITD sludge was mixed with anaerobic sludge from Pen-y-bont sewage treatment plant to increase the solids content of the digester. Operation of the ITD at a B_v of 1.24 g COD l^{-1} d^{-1} (2.8 d HRT) then recommenced on day 78 until day 247.

Anaerobic granules for the UASB were obtained from BPB Paperboard Davidson Mill, Aberdeen. This was a paper pulp processing plant, and therefore the granules were not adapted to dye degradation. The 5 l UASB was filled with 2.5 l of granules and operated continuously from day 150 with a prior 5 day start-up. From days 150-201 it was fed at

a 1 d HRT (B_v: 3.47 g COD l^{-1} d^{-1}), from days 201-235 at a 2 d HRT (B_v: 1.74 g COD l^{-1} d^{-1}) and from days 235-247 at a 1.73 d HRT (B_v: 2.01 g COD l^{-1} d^{-1}).

The 10 l aerobic stage was filled with activated sludge from Pen-y-bont sewage treatment plant on day 183 and fed with effluent from both anaerobic reactors. Solids were recycled every eight hours. It was reseeded on day 193 due to low MLSS of the previous sludge and then operated through until day 247. The HRT of the activated sludge stage fed by the ITD and UASB was 1.24 d, 1.81 d and 1.69 d when the UASB HRT was 1 d, 2 d and 1.73 d respectively. The majority of results were obtained at an aerobic HRT of 1.81 d, subsequent to reseeding. At this HRT the contributions from the ITD and UASB to the aerobic stage were 3.04 and 2.5 l d^{-1} respectively. The settler had a HRT of 0.47, 0.68 and 0.63 days at UASB HRTs of 1, 2 and 1.73 days respectively. The layout of the rig containing the 5 l UASB, ITD and aerobic stage can be seen in Figure 4.1. The nutrient concentrations in the STE were found previously to be in excess of those required by the anaerobic stage (Section 3.4.3). Therefore it was thought some nutrients would be carried over to the aerobic stage, eliminating the requirement for nutrient additions.

4.2.2 Analysis.

The TSS and VSS of ITD sludge were measured prior to seeding the ITD and after day 77. The TSS and VSS were determined prior to reseeding the ITD with additional sludge, and the measurement repeated after day 247. The TS and VS of the UASB granules were measured prior to seeding and subsequent to day 247. The MLSS of the aerobic stage was measured several times a week.

Samples of STE were taken prior to the point of entry to the anaerobic reactors. ITD and UASB effluent were taken from the effluent ports. Infrared spectrometry was carried out on STE and ITD effluent. The COD, TOC and TOD of ITD, UASB and final effluent were measured. Samples of ITD contents for determination of VFA, BA and pH were taken from port 2 until day 29. However, on day 30 a pH probe was put on-line at port

B (Figure 2.1). From this day samples were taken at port 5 rather than port 2 in order to check the calibration of the pH probe. The internal and effluent VFA concentrations of the ITD were measured to determine whether there was any difference between the two measurements. The VFAs, BA and pH of the UASB effluent were also determined. The BOD of mixed anaerobic effluent (2 d UASB HRT) and final effluent was measured.

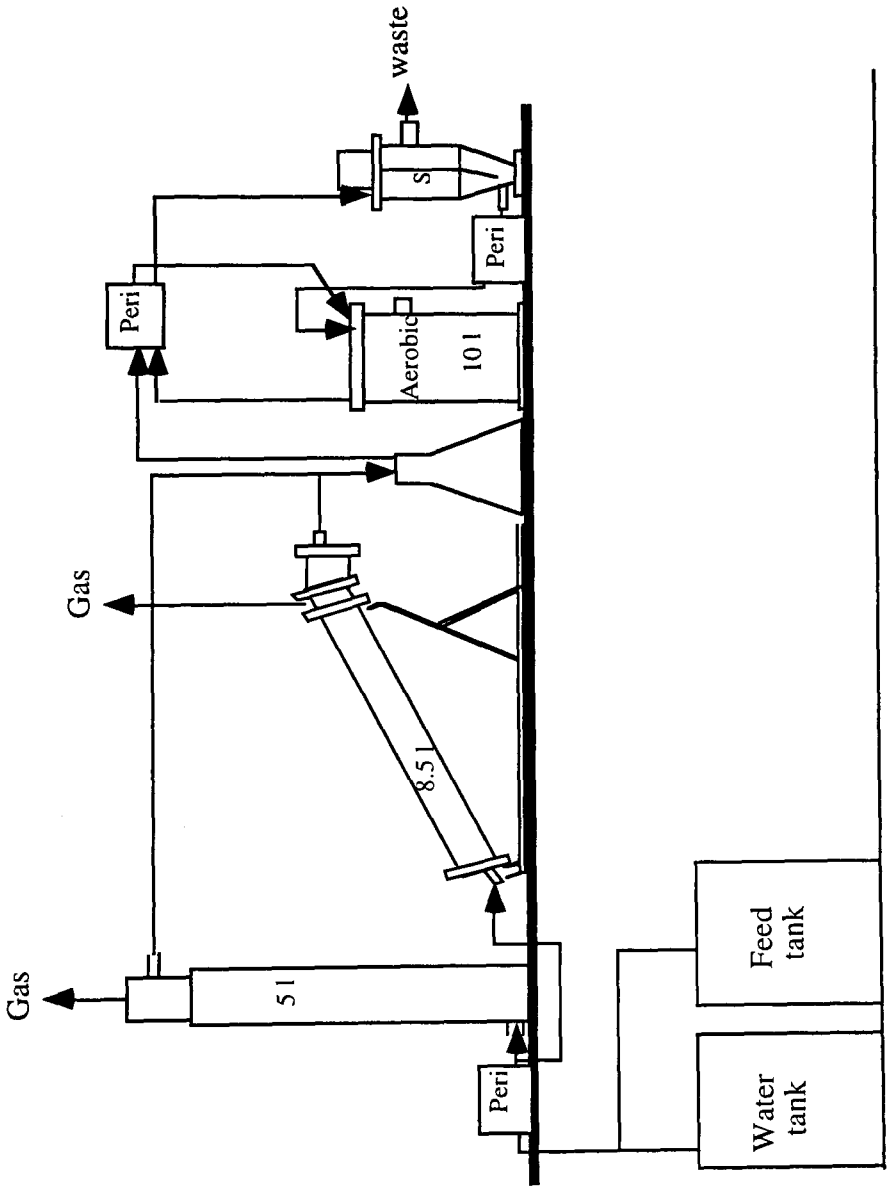


Figure 4.1. Layout Of Rig Containing ITD, UASB, Aerobic Tank And Settler

The absorption spectra of anaerobic and final effluent were examined for comparison with that of the STE (Figure 3.2). True and apparent colour were also measured and compared

with the STE (Table 3.4). The quantity and composition of the biogas produced from the anaerobic digesters was determined.

4.3 Results and Discussion.

Results of ITD performance from days 1-77 after 3 HRT can be seen in Table 4.1. Results from anaerobic and aerobic treatment stages from days 78-247 can be seen in Table 4.2. The mean results for the aerobic stage at all HRTs are presented in this table, the majority being at a 1.81 d HRT. The percentage COD and BOD removals in both tables were calculated from the mean results.

Table 4.1 ITD Digester Performance From Days 1-77.

Analysis	Days	Mean	SD	n
BA (mg $CaCO_3$ l^{-1})	1-28 (3.5d HRT)	1362	(193)	16
	40-69 (2.8d HRT)	1167	(162)	18
	76 (1.4d HRT)	1375		
pH	1-28 (3.5d HRT)	7.4	(0.2)	16
	40-69 (2.8d HRT)	7.0	(0.2)	18
	76 (1.4d HRT)	7.0		
COD (mg l^{-1})	1-28 (3.5d HRT)	3300	(1058)	11#
	40-69 (2.8d HRT)	2026	(709)	17
	76 (1.4d HRT)	2291		
COD removal (%)	1-28 (3.5d HRT)	5		
	40-69 (2.8d HRT)	42		
	76 (1.4d HRT)	34		
BOD (mg l^{-1})	70	422		1#
BOD removal (%)	70	62.6		
COD:BOD	70	4.8:1		
TVFA (mg l^{-1})	1-28 (3.5d HRT)	516	(151)	16
	40-69 (2.8d HRT)	607	(137)	20
	76 (1.4d HRT)	914		
CH_4 (%)	1-28 (3.5d HRT)	28.3	6.7	15
	40-69 (2.8d HRT)	62	9.2	20
	76 (1.4d HRT)	66.8		1

SD-standard deviation, n-no. of samples

#each n is the average of three replicates

Table 4.2 Anaerobic Effluent And Final Effluent Parameters From Days 78-247.

Analysis	ITD 2.8d HRT			UASB 1d HRT			UASB 2d HRT			Final		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
BA (mg l^{-1} $CaCO_3$)	1578	(138)	42	2033	(222)	9	1213		1			
pH	7.2	(0.2)	42	7.3	(0.2)	9	7.1		1			
COD (mg l^{-1})	2174	(450)	69#	2684	(617)	17#	2243	(467)	10#	1641	(246)	19#
COD removal (%)	37			23			35			16		
*BOD (mg l^{-1})	324	(86)	2#				324	(86)	2#	143	(101)	2#
BOD removal (d 137, 237) (%)	71						71			16		
COD:BOD	6.7:1						6.9:1			11.5:1		
TOC day 199** (mg l^{-1})	1587		1#	1012		1#				698		1#
TOD day 208 (mg l^{-1})	2250		1#	2170		1#						
TVFA (mg l^{-1})	353	(233)	71	278	(357)	17	401	(347)	10			
CH_4 (%)	68.3	(7.9)	49	65.2	(5.2)	10	66.6	(0.7)	2			

*calculated from mixed effluent samples, therefore values for ITD and UASB are presented as identical.

** Aerobic TOC is obtained at a 1.24 day HRT

each n is the mean of 3 samples

The ITD was seeded with 11.4 g TSS l^{-1} reactor and 8 g VSS l^{-1} reactor. When the reactor was emptied subsequent to day 77 the TSS and the VSS were 16.7 and 10.2 g l^{-1} of reactor respectively, an increase of 46 and 28% respectively. Therefore some growth of the anaerobic biomass had occurred indicating that it could tolerate this STE. The higher percentage increase in TSS indicates either the deposition of material that was not combusted at 500°C or a source of error in measurement. The ITD was reseeded prior to day 78 with 20.4 g TSS and 13 g VSS l^{-1} reactor. However, this did not settle well initially, with the result that some solids were lost. When the TSS and VSS were measured subsequent to day 247, 17.6 g TSS and 11.2 g VSS l^{-1} of reactor were present, a decrease of 14% for both. The similar decrease for both TSS and VSS from days 78-247 indicates that the difference observed subsequent to days 1-77 was attributable to error. The sludge loading rate (B_x) in the ITD from day 28-69 and 78-247 was 0.12 and 0.11 g COD g^{-1} VSS d^{-1} (B_v: 1.24 g COD l^{-1} d^{-1}; 2.8 d HRT) based on the VSS present subsequent to days 77 and 247 respectively. The sludge loading rates were therefore similar at a 2.8 d HRT in both operating periods. At 3.5 and 1.4 d HRTs the ITD B_x values were 0.097 and 0.24 g COD g^{-1} VSS d^{-1}.

The 5 l UASB had an initial TS and VS of 82 and 68 g l^{-1} reactor. When the UASB was emptied at the end of the experimental period it was found that the TS and VS had fallen to 52 and 40 g l^{-1} reactor, a decline of 37% and 41% respectively. This exceeded the quantity lost from the ITD and was due to washout of granules from the reactor, attributable to incomplete separation at the 3-phase separator. It was therefore concluded that the ITD was superior to the UASB in retaining sludge. The B_x in the UASB was 0.087 g COD g^{-1} VS d^{-1} (B_v: 3.47 g COD l^{-1} d^{-1}), 0.043 g COD g^{-1} VS d^{-1} (B_v: 1.74 g COD l^{-1} d^{-1}) and 0.05 g COD g^{-1} VS d^{-1} (B_v: 2.01 g COD l^{-1} d^{-1}) at a 1, 2 and 1.73 d HRT respectively, based on the sludge present at the end of the operating period. Therefore higher sludge loading rates were obtained in the ITD at the 2.8 d HRT than in the UASB at any HRT. However, the UASB HRTs were shorter, at 1-2 days, by comparison with the ITD (2.8 d HRT; Section 4.2.1), and higher B_v values were obtainable in the UASB due to the higher concentration of biomass present. This meant that larger quantities of STE could be treated.

4.3.1 COD and BOD.

The percentage COD removal by the ITD and UASB (Tables 4.1 and 4.2) was based on the COD of STE given in Table 3.4. The COD of the STE and ITD effluent for days 1-77 can be seen in Figure 4.2. There was some contamination of samples at the 3.5 d HRT (days 1-28) from the screw-caps. This was resolved by the time the B_v was increased to 1.24 g COD l^{-1} d^{-1}. However, it made the COD results for d 1-28 unreliable, resulting in apparently poor COD removal (Table 4.1). It was assumed that contamination affected all samples equally. Therefore the percentage COD removal was recalculated from the daily STE COD results for this HRT rather than from the mean STE COD (Table 3.4). This gave a mean COD removal of 46%. When the COD removal for days 40-69 was calculated from the daily results a 38% removal was obtained. This differed slightly from the 42% given in Table 4.1. However, since the error associated with COD measurement is high, this difference was unlikely to be significant. When the recalculated COD value for days 1-28 was compared with the COD removal at 2.8 and 1.4 d HRTs it was seen that the percentage COD removal was lower at shorter HRTs, as expected.

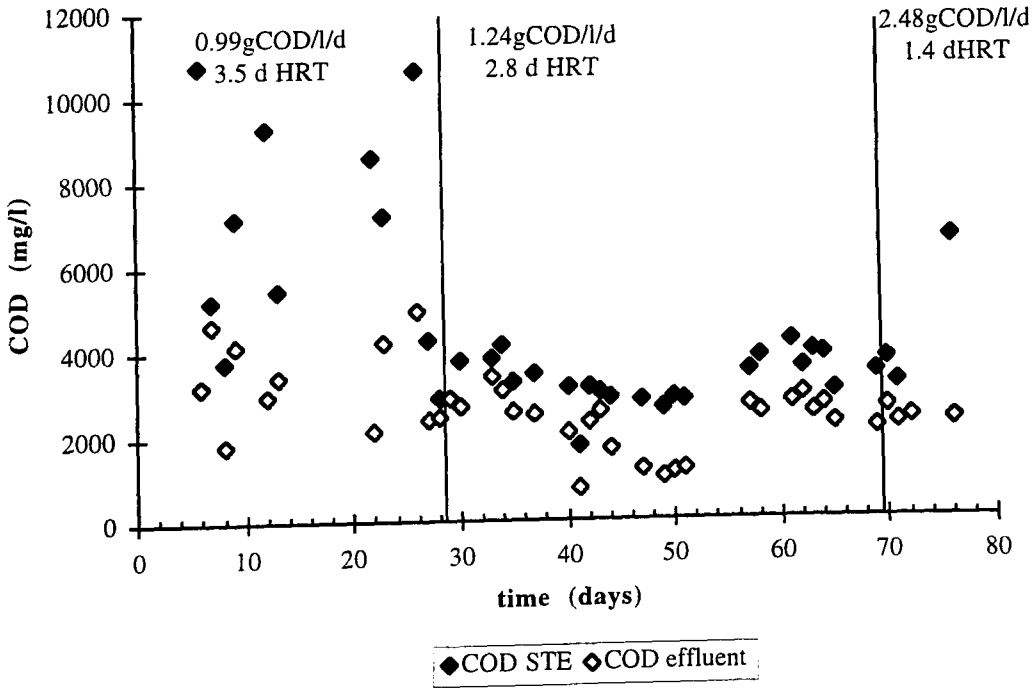


Figure 4.2 COD Of STE And ITD Effluent vs Time.

When the results from d 78-247 were examined it was seen that the majority of COD removal occurred in the anaerobic stage, with a smaller amount occurring aerobically (Table 4.2). This proved that the anaerobic stage reduced the load to the activated sludge stage. The percentage COD removed aerobically was based on the anaerobic COD contributions of both digesters. The percentage COD removal from the ITD (2.8 d HRT) and the UASB (2 d HRT) was similar, at 37 and 35% respectively. A 30% COD removal was achieved when the UASB COD removal (1 d HRT) was calculated from the daily STE measurements rather than from the value given in Table 3.4. This was in excess of the 23% calculated from the mean STE COD and can be attributed to the variation in COD measurement. At a 2 d UASB HRT the values determined from the mean and daily STE results were similar at 35 and 32% respectively. It was seen that the percentage COD removal in the UASB at a 2 d HRT was similar to that achieved in the ITD at a 2.8 d HRT. However the sludge loading rates obtained in the UASB were lower (Section 4.3). The percentage of COD and TOD removed by the ITD was comparable at 37 and 35% respectively while the TOC removal was lower, as expected, at 24%. The TOC removal by the UASB (1 d HRT) was 52% and therefore did not compare well to the 23% COD removal. The TOD removal was 37%, comparing better with the 30% COD removal calculated from the daily STE measurements than the 23% calculated from the mean STE COD.

The aerobic COD removal was calculated from the different contributions from the UASB (2 d HRT) and ITD (Section 4.2.1). An overall COD removal of 53% was achieved after combined anaerobic-aerobic treatment. The theoretically readily removable COD, calculated from the theoretical COD of starch and acetic acid (Section 3.2.3), comprised 65% (2250 mg l^{-1}) of the measured COD. The overall COD removal was lower than this, showing that not all the readily removal COD was eliminated by combined anaerobic-aerobic treatment. This was verified by the presence of 143 mg l^{-1} BOD in the final effluent of combined treatment. An overall BOD removal of 87% was achieved (Table 4.2), 71% of which occurred anaerobically.

The initial COD:BOD of the STE was 3.1:1 (Table 3.4). The COD:BOD of the ITD effluent on day 70 (Table 4.1) was 4.8:1 based on the COD of days 40-69 and the BOD on day 70. The COD:BOD of the anaerobic effluent between days 78-247 was higher at 6.7:1 and 6.9:1 for the ITD and the UASB (2 d HRT) respectively. Hence anaerobic treatment removed more biodegradable material in days 78-247. The COD:BOD of the final effluent was 11.5:1. Therefore the COD:BOD ratio increased during treatment, as expected, due to removal of biodegradable material. The F:M for the ITD and UASB in Experiment 1 was 0.032-0.079 and 0.014-0.028 g BOD g^{-1} TV(S)S d^{-1} respectively. This was lower than the normal 0.5-1 g BOD g^{-1} TV(S)S d^{-1} (Section 1.5.2.1.2) but tested the upper tolerance limits of both digesters as will be explained in Section 4.3.3.

The incomplete removal of COD and BOD may be due to a number of factors: inhibition, due either to the dye or the degradation products; insufficient mixing, leading to poor contact of the wastewater with the biomass; or the HRT of the anaerobic digester may have been too short to allow complete degradation to occur. At long HRTs mixing within UASBs tends to be poor and a very large reactor would be required for treatment of appreciable quantities of industrial textile effluent. The reported lack of inhibition of anaerobic systems by this dye makes it an unlikely cause of the poor removal, although the concerns of Carliell *et al.* (1995) regarding amine toxicity to anaerobic bacteria are worthy of consideration.

4.3.2 Gas Measurements.

Some technical difficulties with gas collection were experienced prior to day 40, accounting for the low percentage methane in days 1-28, but biogas composition was relatively constant throughout the remainder of the operating period. Due to the limited number of steady state results for the 1.4 d HRT the gas yields were merely an indication of whether the ITD was working well and could not be compared statistically. The rate of gas production in the ITD from days 57 to 67 was 88 mls l^{-1} reactor d^{-1}, corresponding to a methane yield of 0.105 l CH_4 g^{-1} COD removed (SD: 0.05; n: 11). The gas production on day 76 was 101 mls l^{-1} reactor d^{-1} giving a methane yield of 0.08 l CH_4 g^{-1}

COD removed. These values were below the theoretical methane yield of 0.35 l CH_4 g^{-1} COD removed at STP (Section 1.5.2.1). The reactor was leak-tested regularly to ensure that the low yield was not due to gas losses to the atmosphere, but no leaks were detected.

The rate of gas production by the ITD from days 81-213 was 103 ml l^{-1} reactor d^{-1} while that of the UASB from days 206-213 (2d HRT) was 115 ml l^{-1} reactor d^{-1}. These rates of gas production gave a methane yield of 0.15 (SD: 0.08; n: 125) and 0.12 (SD: 0.01; n: 10) l CH_4 g^{-1} COD removed for the ITD and UASB respectively. The gas yield of both anaerobic reactors was again below the theoretical methane yield (Section 1.5.2.1). The gas production rates and the methane yields for both digesters were similar. The small volumes of gas produced means that poor mixing may have contributed to the incomplete removal of readily biodegradable COD (Section 4.3.1). Gas yields below the predicted value in a UASB and anaerobic sequencing batch reactor (ASBR) have been reported to be due to utilisation of soluble COD by biomass growth and biogas losses (Angenent and Dague, 1995). Tang *et al.* (1995) attributed the deficit in yield in a UASB to COD being trapped in the sludge blanket or methane being dissolved in the effluent and escaping in that fashion. Competition of SRBs with methanogens can also result in lower methane yields. There were therefore a number of reasons that could account for the measured methane yield being less than the theoretical value.

4.3.3 Bicarbonate Alkalinity and Volatile Fatty Acids.

The bicarbonate alkalinity measured within the anaerobic reactors was 1167-2033 mg l^{-1} as $CaCO_3$ (Tables 4.1 and 4.2). It therefore exceeded the minimum recommended concentration of 1000 mg l^{-1} as $CaCO_3$ (Section 1.5.2.1). It was seen from the standard deviations that little fluctuation occurred throughout the experimental period. The bicarbonate alkalinity did not fall below 500 mg l^{-1} $CaCO_3$, the alkalinity at which pH falls (Fannin, 1987). The stability of the pH in both anaerobic reactors, as evidenced by the low standard deviations (Tables 4.1 and 4.2), indicated that the buffering capacity was adequate for operation of this reactor.

When the VFA concentrations within the ITD and in the ITD effluent were analysed it was found that the latter appeared to be lower than former (Table 4.3). However, these differences were not statistically significant and hence either sample type could be used for determination of VFA concentration.

Table 4.3 Concentration Of Individual VFAs Inside The ITD ('Internal') And In The ITD Effluent At A B_v Of 1.24 g COD l^{-1} d^{-1} (Days 40 To 69).

	Internal (mg l^{-1})			ITD effluent (mg l^{-1})		
Acid type	mean	SD	n	mean	SD	n
acetic	492	(121)	20	461	(111)	20
propionic	103	(39)	20	83	(34)	20
i-butyric	2.7	(1.0)	20	2.7	(0.9)	20
n-butyric	5.3	(1.7)	20	4.5	(1.6)	20
i-valeric	3.6	(1.7)	20	3.6	(1.4)	20
n-valeric	0.4	(0.2)	20	0.4	(0.3)	20
Total	607	(137)	20	555	(130)	20

The VFA concentrations in the ITD effluent for days 1-77 were compared with the concentrations in the STE (Figure 4.3). It was seen that the TVFAs in the ITD were not greatly affected by the absence of acetate in the STE (Section 2.5.2; Figure 4.3). T-tests found no significant difference in acetic acid or TVFA concentration between the periods when acetic acid was present and absent in the STE. However, a significant difference was found for propionic acid (99.9% CI) in both the internal and effluent samples. As acetic acid was the dominant VFA and there was no significant difference in TVFA concentration, it was concluded that the absence of acetic acid in the STE did not affect the ITD.

Other VFAs were detected in both anaerobic digesters in addition to acetic acid, the only VFA normally present in STE, as illustrated by Table 4.3. The concentration of propionic acid in the ITD was low at a B_v of 0.99 g COD l^{-1} d^{-1} (Figure 4.3). When the B_v was increased to 1.24 g COD l^{-1} d^{-1} (days 28-69) the concentration of propionic acid rose. At a 1.4 d HRT (d 69-77) the TVFA rose from ~600 mg l^{-1} to ~900 mg l^{-1} (Table 4.1). Increases in propionic acid or in TVFA concentrations indicate inhibition or other

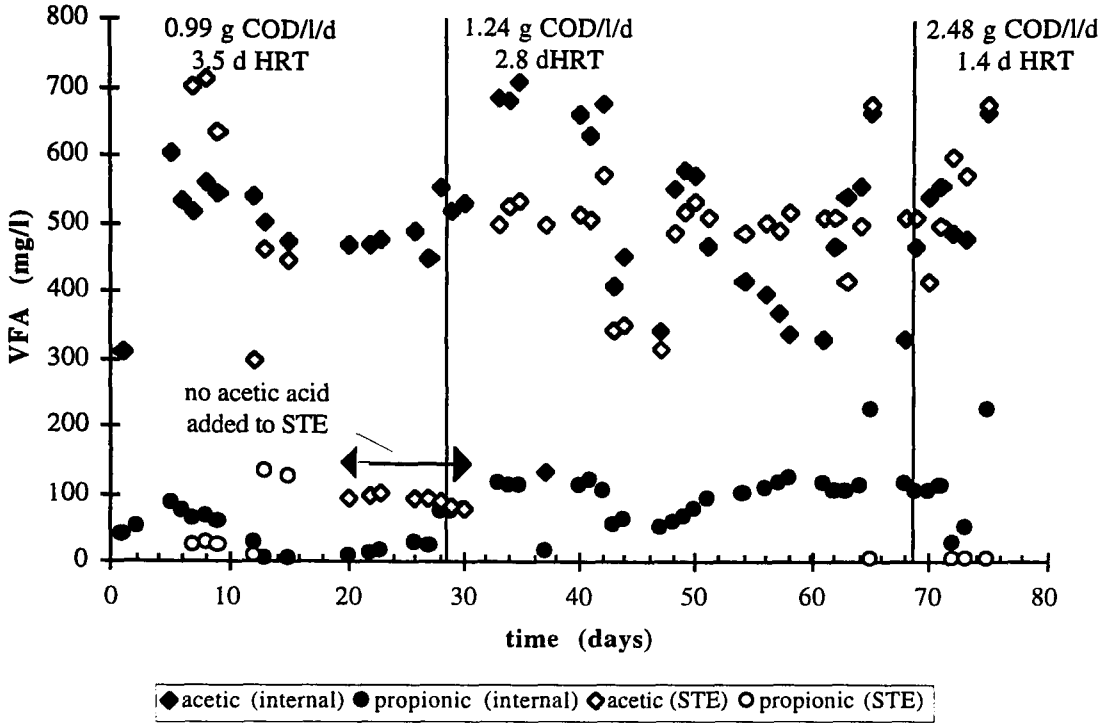


Figure 4.3 Concentration Of Acetic And Propionic Acid In STE And ITD ('Internal') For Days 1-77.

problems within the reactor (Section 1.5.2.1). Therefore the tolerance limits of the ITD had been reached. It was found from the data in Table 4.1 that the TVFA:BA was in excess of that found in stable systems (Section 1.5.2.1) at 0.38-0.66. However, given the good COD removal at a B_v of 1.24 g COD l^{-1} d^{-1}, this loading rate was used from d 78-247 despite the TVFA:BA of 0.52. Between days 78-247 a mean 279 mg l^{-1} acetic acid (SD: 156; n: 66) and 55 mg l^{-1} propionic acid (SD: 49; n: 65) were present in the ITD (B_v: 1.24 g COD l^{-1} d^{-1}). The TVFA:BA was 0.22, from the data in Table 4.2. Although these figures did not indicate that the ITD was stressed, the TVFAs increased from day 78-247 (Figure 4.4) indicating intolerance. This increase was in excess of that observed between days 28-69 (Figure 4.3).

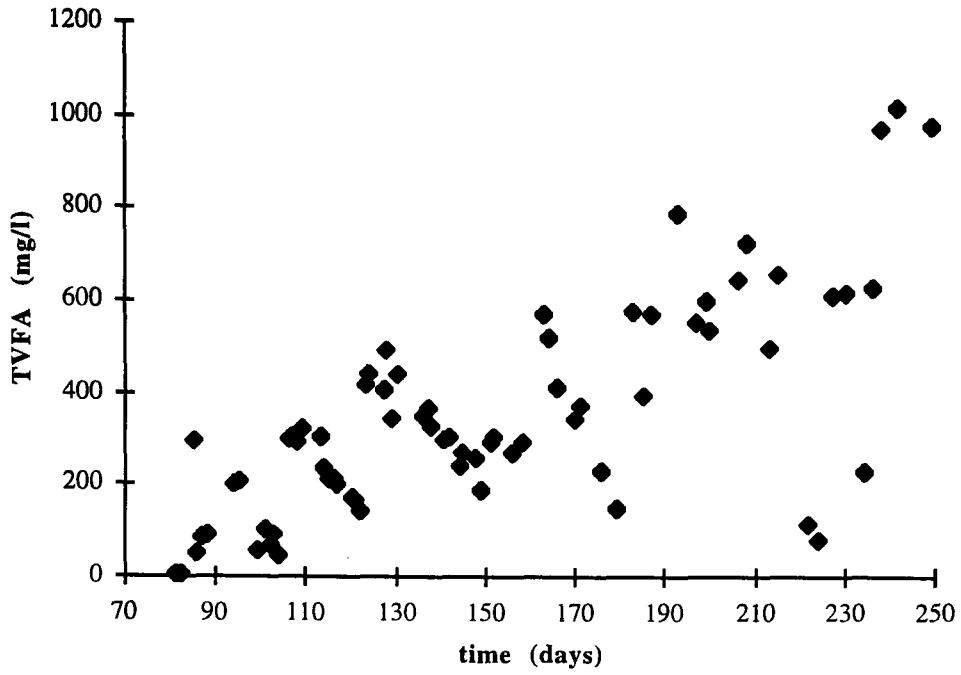


Figure 4.4 TVFA Concentration In The ITD (Days 78-247).

The concentration of TVFAs in the UASB was low initially but increased after day 180 from approximately 200 mg l^{-1} to over 1000 mg l^{-1} (Figure 4.5), indicating intolerance. Therefore the HRT was increased to 2 days on day 201. By day 235 the TVFA concentrations had fallen below 200 mg l^{-1} and so the HRT was decreased to 1.73 days. The TVFAs in the UASB did not increase between day 235 and 247 showing that the reactor was not stressed at this HRT. Given the changes in TVFA concentrations, the TVFA:BA was not calculated for the UASB. The TVFA concentrations indicated that the maximum tolerance of the UASB lay between 2.01-3.47 g COD l^{-1} reactor d^{-1} when treating this STE.

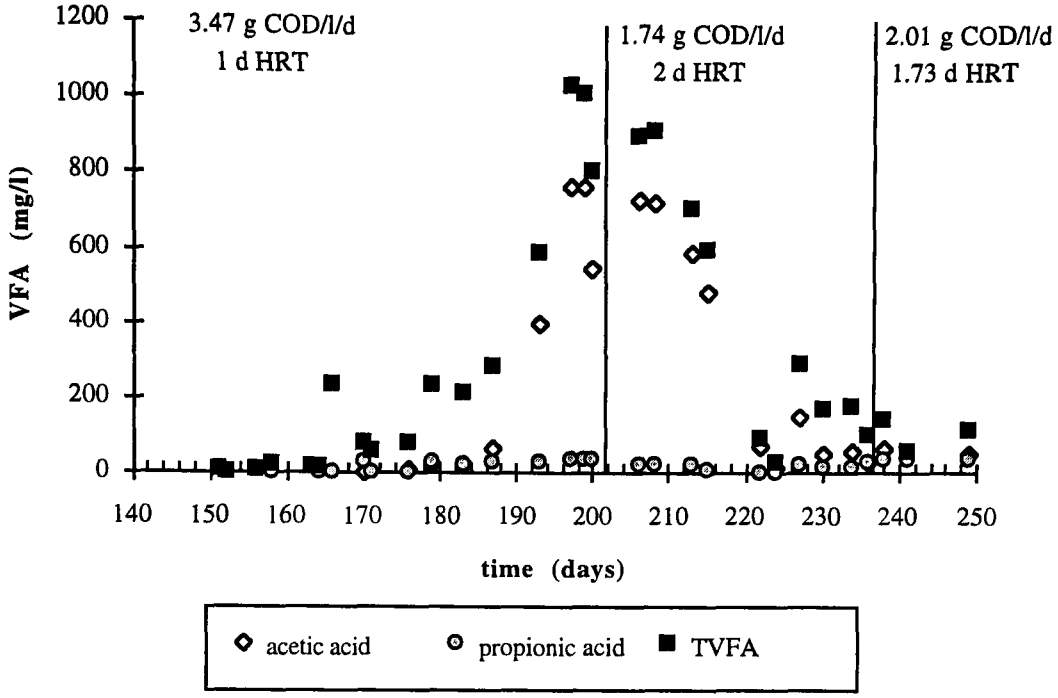


Figure 4.5 Acetic, Propionic And TVFA Concentrations In Effluent From The 5 l UASB From Days 150-247.

4.3.4 Colour.

The absorption spectrum of a 20-fold dilution of STE and anaerobic effluent from the ITD can be seen in Figures 3.2 and 4.6 respectively (B_v: 0.99 g COD l^{-1} d^{-1}). The peak between 440 and 600 nm corresponding to the presence of dye (Figure 3.2) was removed by the ITD at a B_v of 0.99 and 1.24 g COD l^{-1} d^{-1}. It was replaced by a smaller peak around 440 nm (Figure 4.6). However, following the step loading to 2.48 g COD l^{-1} d^{-1}, the colour peak was not removed although its intensity decreased. Thus a HRT of 1.4 days in the ITD was insufficient to decolourise the dye. Carliell *et al.* (1996) noted that rates of decolourisation were inversely proportional to the initial dye concentrations. Therefore, longer HRTs, impractical as they may be in terms of quantities of effluent treated, result in better colour removal.

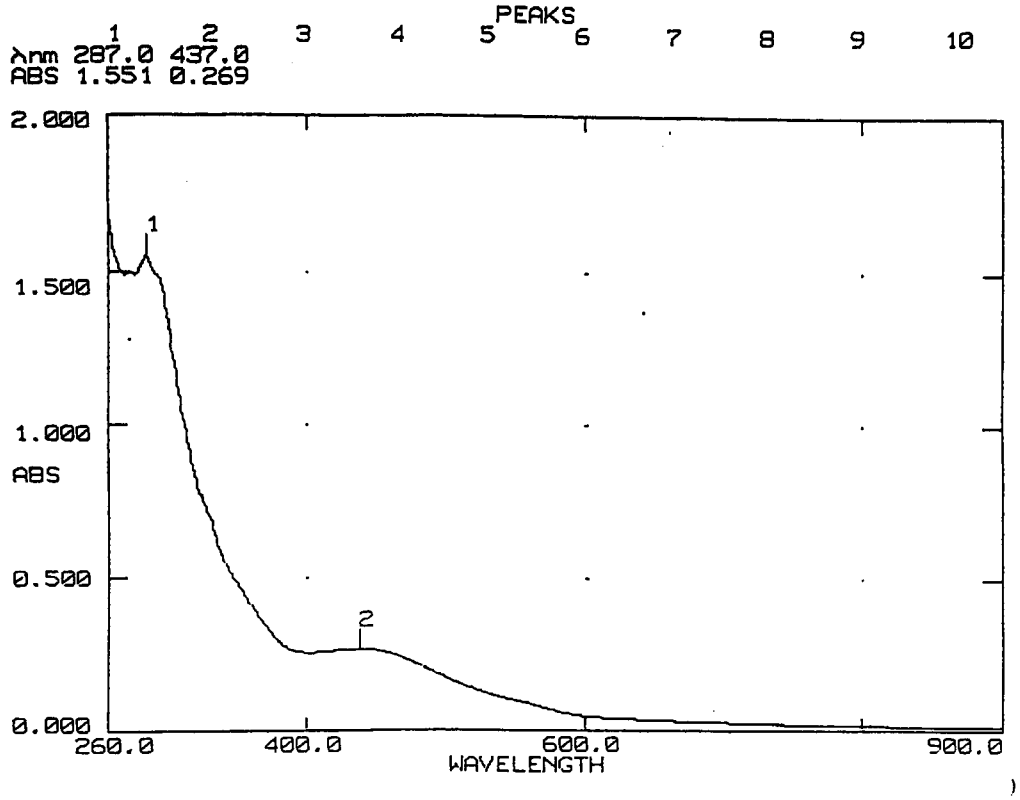


Figure 4.6 Absorption Spectrum Of A x20 Dilution Of ITD Effluent On Day 27 (B_v: 0.99 g COD l^{-1} d^{-1}).

The apparent and true colour measurements of ITD, UASB and final effluent can be seen in Table 4.4. Colour measurements for the STE are given in Table 3.4. The colour of effluent entering the aerobic stage was calculated from the contributions of the different volumes of UASB and ITD effluent entering this stage at a 1.81 d aerobic HRT (Section 4.2.1), as was the mean anaerobic colour removal. The percentage aerobic colour removal was calculated from the mean results. Overall, 69 and 68% apparent and true colour removal respectively was achieved by means of combined treatment while up to 79% of apparent colour and 78% of true colour were removed anaerobically. The UASB gave better colour removal than the ITD. The superior colour removal by the UASB made it a more effective reactor for treatment of this STE. There was a slight increase in colour in the final effluent compared to the anaerobic effluent. This could be due to the effect of the increase in pH in the aerobic stage on colour (Section 4.3.5), or oxidation of some of some of the anaerobic reduction products as suggested by Knapp and Newby (1995). In spite of this, the role of aerobic treatment in amine breakdown and COD removal means it can be useful in textile effluent treatment subsequent to anaerobic digestion. This was

evidenced by the fact that 16% of COD was removed aerobically between days 78-247 (Table 4.2). However, the combined treatment effluent still had an average true colour of 1.78 abs units (Table 4.4). It would therefore need to be highly diluted or treated further prior to discharge.

Table 4.4 Apparent (App.) Colour, True Colour And Percentage True Colour Removal Of ITD, UASB And Final Effluent (days 222, 229).

Analysis	ITD 2.8d HRT			UASB 2 d HRT			Final		
	mean	SD	n	mean	SD	n	mean	SD	n
App. colour (abs units)	2.07	(0.2)	2	1.19	(0.04)	2	1.74	(0.25)	2
% Reduction	63	(4.6)	2	79	(0.06)	2	-4.3		
True colour (abs units)	2.11	(0.16)	2	1.23	(0.06)	2	1.78	(0.25)	2
% Reduction	62	(3.6)	2	78	(0.7)	2	-3.9		

4.3.5 Aerobic Stage.

The aerobic tank was filled with activated sludge containing 2.41 g l^{-1} of MLSS and 2.26 g l^{-1} VSS on day 193. This declined over the operating period to 1.32 and 0.94 g l^{-1} respectively. Therefore no settled solids were disposed of to waste. This gave a F:M of 0.07-0.14 g BOD g^{-1} MLSS d^{-1} based on biomass present at the beginning and end of the experiment respectively. This was within the normal range of 0.1 to 0.5 g BOD g^{-1} biomass d^{-1} cited for aerobic systems (Section 1.5.2.2). A mean of 27.8% (SD: 6.1; n: 5) of MLSS was lost from the settling test as unsettled solids, giving a sludge age of under 4 days. As the biomass in the aerobic tank declined over the operating period, the rate of biomass growth was less than the rate of loss of solids in the settling tank effluent. The pH within the activated sludge tank was 8-9, attributable to the large quantity of NaOH used to hydrolyse the starch. The anaerobic digestion process maintained a pH around neutral due to the production of VFAs but under aerobic conditions the VFAs volatilised or were metabolised, resulting in a rise in pH. At this pH most of the protozoa die (H. Buckland, Yorkshire Water, pers. comm.). Therefore high pH was a possible cause of poor biomass growth.

4.3.6 Amines.

When samples of STE and ITD effluent were scanned using IR spectrometry a number of peaks were observed as can be seen in Figures 4.7(a) and (b). Peaks were identified using the guide given by Williams and Fleming (1995).

Strong peaks were observed in both the STE and ITD effluent samples at about 3400 cm^{-1}, attributable to the presence of free -OH. Amines can be detected between 3500-3300 at medium intensities, and so could have been hidden by the -OH peak. A small peak was observed in both samples just below 3000 cm^{-1}, which could have been caused by $-CH_3$, CH_2 or possibly intramolecular 'chelate' H-bonded -OH. The presence of acetic acid in both samples could have given rise to these peaks. In the ITD effluent a peak was noticeable at about 2600 cm^{-1}. This was in the region where $\rangle NH_2^+$, $=\overset{+}{N}H$ and $\overset{\diagdown}{\underset{\diagup}{}}NH^+$ absorb. Another possible substance causing absorption in this area was $\overset{O}{\overset{\|}{\underset{\diagup}{\diagdown}}}P-OH$. No obvious peak was observed in the STE in this region, although the STE contained phosphate compounds. The peaks could therefore be due to the presence of amines in the ITD effluent.

Vibrations due to whole molecules give rise to absorption bands at low energy, below 1500 cm^{-1}. Therefore localised vibrations below this can be difficult to identify. Nevertheless an attempt was made to identify the major peaks in this region. Peaks were observed at approximately 1600 and 1400 cm^{-1} in both the STE and ITD effluent. At 1600 cm^{-1} a wide range of molecules is detected including $-NH_2$, and $-NH_3^+$. Another group detected between 1490 and 1580 is $\rangle NH$. Aromatic rings can also be detected between 1500 and 1600 cm^{-1}. At 1400 cm^{-1} the most likely possibilities were -O-H and $-SO_2O-$. In the STE the peak at 1400 cm^{-1} had a higher transmittance than that at 1600 cm^{-1}, whereas in the ITD effluent the peaks had similar transmittance. This is

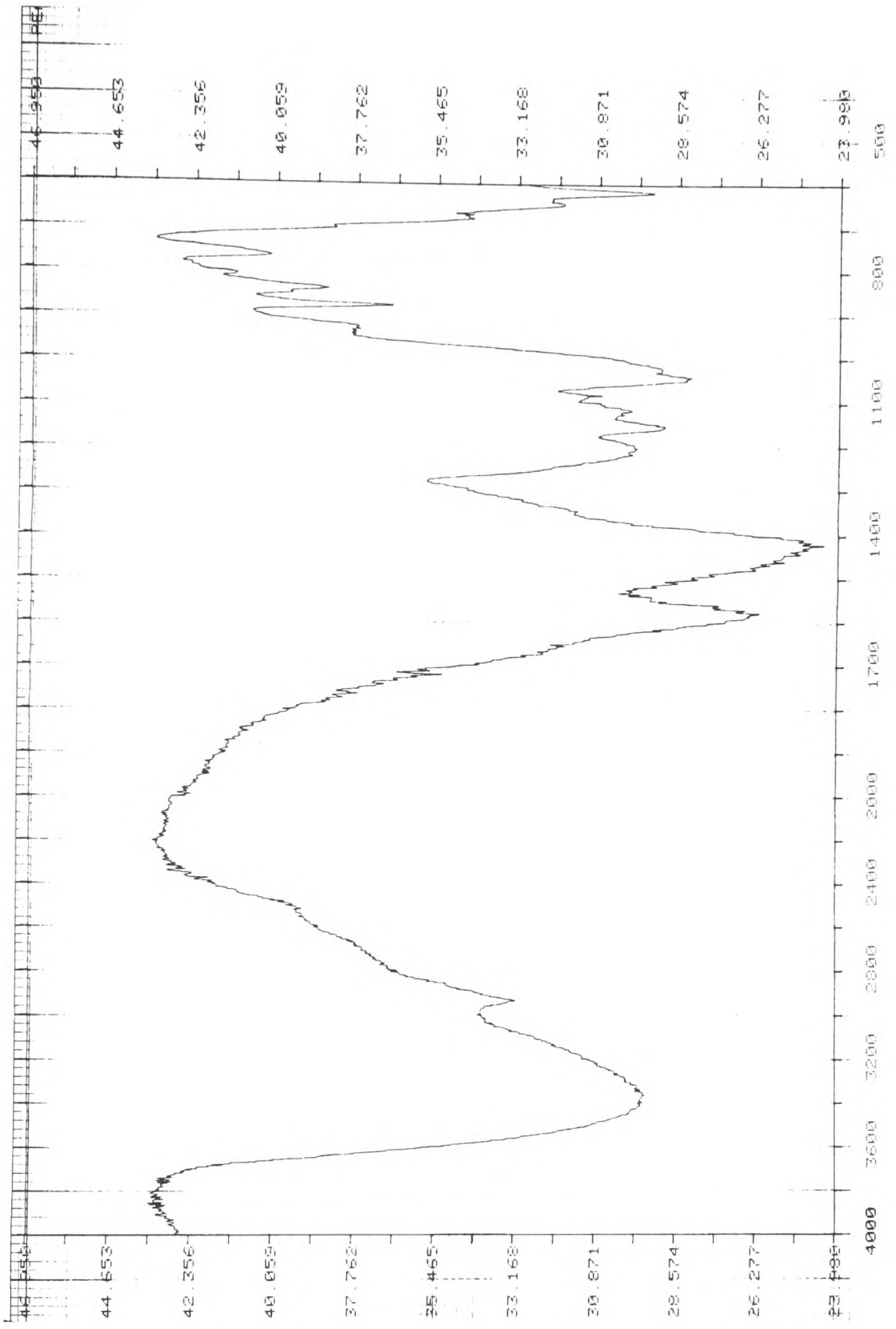


Figure 4.7(a) Infrared Spectrum Of STE (x axis-cm^{-1}, y axis-% transmittance).

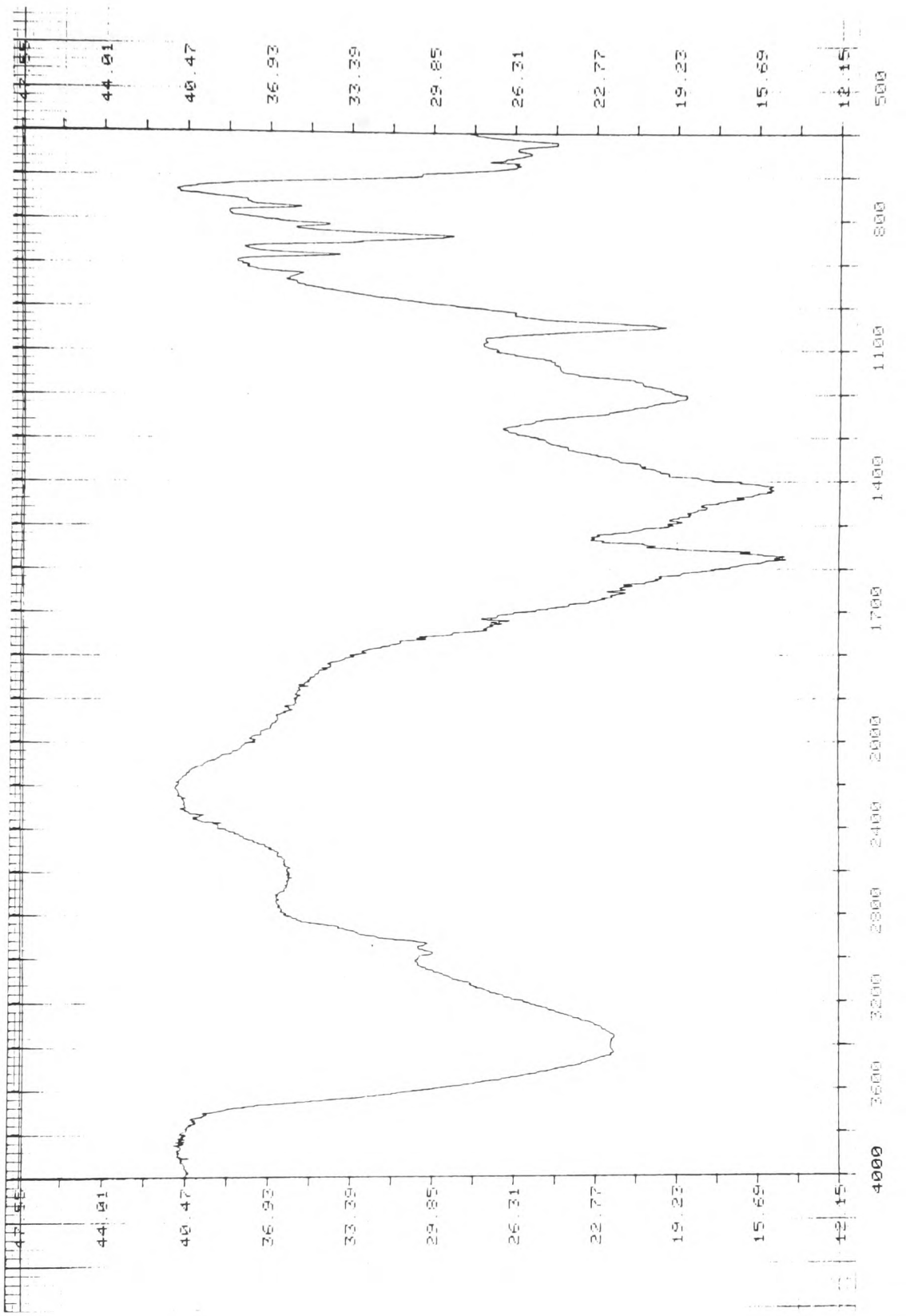


Figure 4.7(b) Infrared Spectrum Of ITD Effluent (x axis-cm^{-1}, y axis-% transmittance).

indicative of changes occurring by means of anaerobic degradation. Azo bonds do not vibrate strongly in the infrared region and are located between 1500-1400 cm^{-1}, which means they can be confused with entire-molecule vibrations. However, it is possible that the peak at 1400 cm^{-1} is at least partly attributable to azo bonds and that the reduced intensity of this peak by comparison with that at 1600 cm^{-1} in ITD effluent may be due to cleavage of these bonds. Alternatively it is possible that the peak at 1600 cm^{-1} increased in intensity after anaerobic treatment due to formation of amines which then absorbed at this wavelength.

A peak was observed at 1200 cm^{-1} in both samples. This could have been due to a range of groups, the most likely of which were  or $-SO_2O-$ arising from the sulphonic groups present on the dye molecule. A peak was present at about 1050 cm^{-1} in both samples, which was most likely to be attributable to C-O. A large number of small peaks were present between 700-1000 cm^{-1} in both samples. Given their number, small intensity, and presence in both samples, it was not necessary to identify them. However, peaks in this area are normally attributable to C-H bonds and substituted benzene rings. Given the nature of the STE, either or both of these could have given rise to these peaks.

The overlap between peaks and the number of bonds that can give rise to vibration at given wavelengths make it difficult to distinguish some bonds. Many bonds, such as those present in starch and acetic acid, are cleaved during anaerobic digestion and would thus be expected to give rise to differences between STE and ITD IR spectra. This makes it difficult to isolate changes due to dye degradation. For these reasons, although the peak in the ITD effluent at 2600 cm^{-1} indicated that amines associated with colour removal might be present after anaerobic degradation of this dye, and the peaks at 1400-1600 cm^{-1} may also have reflected degradation of azo bonds to amines, it was concluded that IR spectrometry is not a very useful method for detecting their presence in treated textile effluent.

4.4 Conclusions.

The ITD appeared adapted to operation at a 2.8 d HRT within two months of start-up as effluent COD and percentage COD reduction in days 40-69 and 78-247 (Tables 4.1 and 4.2) were similar at 2026-2174 mg COD l^{-1} and 42-37% respectively. The COD:BOD was higher in the ITD effluent between days 78-247 compared to that on day 70 at 6.7:1 and 4.8:1 respectively, indicating that more BOD was removed aerobically in the second operating period.

Higher sludge loading rates were obtained in the ITD (2.8 d HRT) compared to the UASB at all HRTs tested although the ITD HRT was longer. The increase in TVFA in the ITD at a B_v of 1.24 g COD l^{-1} d^{-1} from days 78-247 showed that the digester could not tolerate this loading rate for prolonged periods. Higher volumetric loading rates were obtained in the UASB with the optimum B_v lying between 2.01-3.47 g COD l^{-1} d^{-1}. The ITD was superior to the UASB in retaining its sludge, losing only 14% TSS in d 78-247 by comparison with the 37% TS lost by the UASB in d 150-247.

The majority of COD was removed anaerobically. Therefore anaerobic treatment was proved to reduce the COD load to the aerobic stage. The percentage COD removal in the UASB was greater at HRTs in excess of 1 d, with a 2 d UASB HRT giving similar COD removal to that of the ITD at a 2.8 d HRT. In combined treatment (days 78-247), anaerobic digestion removed up to 37% of COD and 71% of BOD with a mean COD removal of 53% and BOD removal of 87% being achieved after aerobic treatment of the anaerobic effluent. Even after aerobic treatment not all of the theoretically readily removable COD was removed.

The concentration of TVFAs in the UASB increased at a 1 d HRT requiring an increase in HRT. This observation, combined with the increased COD removal at longer HRTs, led to the conclusion that for this STE composition UASB retention times in excess of 1 d were required. The mean TVFA in the ITD in days 78-247 was lower than that in days 40-69, with a mean of 353 mg l^{-1} compared to 607 mg l^{-1} although the same loading rate was used in both periods. However, it was seen from Figure 4.4 that the TVFAs

increased in days 78-247, indicating that the ITD was more stressed than previously. Therefore longer HRTs were likely to prove beneficial. These would be less economical, however, as less STE could be treated.

Anaerobic treatment removed up to 78% of the true colour of STE, with the UASB removing more colour than the ITD. At low loading rates (B_v: 0.99-1.24 g COD l^{-1} d^{-1}) use of an ITD was also successful in decolourising the STE. It was found that longer HRTs resulted in better colour removal in the ITD. No colour removal occurred aerobically.

Aerobic treatment removed some additional COD and BOD. Effluent from the combined process operating on this STE still contained 1641 mg COD l^{-1}. Therefore further treatment would be required. The combined treatment effluent was highly coloured, with a true colour of 1.78 absorbance units, and would also require further treatment. The pH of the aerobic stage was relatively high (8-9) which may have reduced the efficiency of operation of this stage.

The UASB was more effective than the ITD in treatment of STE as COD removal at a 2 d HRT was comparable to that of the ITD at a 2.8 d HRT, while the colour removal was superior. The TVFAs did not increase at this HRT, unlike the ITD, and the HRT required was shorter than that of the ITD. This meant that larger volumes of effluent could be treated by the UASB. The principal disadvantage of the UASB was the loss of biomass over time.

It was difficult to tell from infrared spectrometry whether or not amines were generated through anaerobic treatment of STE. However, there were some indications that they might be present in ITD effluent.

CHAPTER FIVE - STEADY STATE TREATMENT AND STEP CHANGES IN TREATMENT OF SIMULATED TEXTILE EFFLUENT WITH A 30 L UASB AND ACTIVATED SLUDGE STAGE.

5.1 Introduction.

A 30 l UASB replaced the two anaerobic reactors used previously in order that sufficient anaerobic effluent be provided to operate a 20 l aerobic stage. In Experiment 2 STE containing the original concentrations of dye, salt and starch (Table 3.4) was fed to the UASB at a 2.5 d HRT. The dye and salt concentrations were then reduced and steady state operation was carried out at a 1.7 d UASB HRT (Expt 3.0). Step changes were enacted at a 1 d UASB HRT by decreasing the dye and starch concentrations (Expts 3.1-3.4). This enabled a range of starch:dye ratios to be investigated. STE that had not been pre-treated anaerobically was fed to the aerobic stage in Expt 3.5.

The performance of the UASB was assessed in all cases. The role of the aerobic stage in combined anaerobic-aerobic treatment of STE was also determined. The results achieved were compared with those obtained by other authors. The effect of altering the starch and dye concentrations was examined along with the effect, if any, of the starch:dye ratios on colour removal. The nutrient carry-over from the UASB to the aerobic stage was examined.

5.2 Methods.

5.2.1 Reactors and Experimental Design.

The UASB was seeded with 10 l of the granules described in Section 4.2.1, which were stored at room temperature prior to use. In Experiment 2 the UASB was fed at a HRT of 2.5 days with dye and salt concentrations of 1.5 g l^{-1} using a x10 concentrate of STE. It was hoped that the long HRT would provide good decolourisation of the dye. The UASB was operated in steady state for 28 days following a 7 day start-up period. Due

to the long HRT a recycle pump was attached to the recycle/sample port at the top of the UASB for the duration of this Experiment to assist mixing within the reactor. Effluent was recycled into the reactor through the feed line. No activated sludge stage was used in conjunction with this Experiment.

Experiment 3 was comprised of Expts 3.0-3.5. In Expt 3.0 the concentrations of dye and salt were decreased to 0.15 g l^{-1} (Table 5.1). The salt was maintained at this concentration thereafter. The UASB was then fed at a 1.7 d HRT from days 1-26 with a x30 concentrate of STE following a 9 day start-up period. Steady state measurements were taken from day 4. On day 26 the HRT was reduced to 1 d, using the same STE, and the recycle removed as the shorter HRT improved mixing. However, an assortment of problems was experienced between days 26-46 including pump failure and coagulation of starch in the highly concentrated feed and therefore no measurements were taken during this period. To solve these problems a number of repairs were carried out and less concentrated STE was used in the feed tank subsequently. Expt 3.0 was affected to a lesser extent by the starch coagulation problems.

Table 5.1 Days Of Experiment 2 and Expts 3.0-3.4.

Experiment / Expt	Days	Dye Conc. (g l^{-1})	Starch Conc (g l^{-1})	NaCl Conc. (g l^{-1})	BA Conc. (g l^{-1})
2	1-28	1.5	1.9	1.5	1.5
3.0	1-26	0.15	1.9	0.15	1.5
problem	26-46	0.15	1.9	0.15	1.5
3.1	46-53	0.075	1.9	0.15	2.0
3.2	53-60	0.075	0.95	0.15	2.0
3.3	60-67	0.15	1.9	0.15	2.0
3.4	67-74	0.15	0.95	0.15	2.0
3.5	1-24	0.075	0.95	0.15	0.5-1.0

Step changes were carried out from day 46 (Expts 3.1-3.4) by altering the dye and starch concentrations. A x15 concentrate of STE was used and each Expt lasted for 7 days (Table 5.1). The concentration of sodium bicarbonate in the water tank was increased from the 1.5 g l^{-1} used for Experiments 1, 2 and Expt 3.0 to give a final concentration of 2 g l^{-1} during Expts 3.1-3.4. In Expt 3.5, carried out subsequent to Experiment 4, STE that

had not been subjected to anaerobic pre-treatment was fed to the 20 l activated sludge stage at a 0.67 d HRT. A x10 concentrate of STE was used and adjusted to pH 7 with sulphuric acid. The dissolved oxygen was controlled in Expt 3.5 (Section 2.2.3).

A 20 l activated sludge tank was used in Experiment 3 from day 14. It was filled with sludge obtained from Coslech, a local treatment plant that treated waste from a local L'Oreal factory in addition to domestic waste. It was reseeded on day 40 and again on day 42. The sludge tended to foam after collection, which was counteracted by use of anti-foam. Control procedures were implemented from day 21 (Expt 3.0) to maintain the pH of the activated sludge tank at around neutral (Section 2.2.3) in order to compensate for the increase in aerobic pH observed in Experiment 1 (Section 4.3.5). Sludge was recycled from the settler every four hours from day 14 in Experiment 3. At a 1.7 d and 1 d UASB HRT the aerobic stage had a 1.14 and 0.67 d HRT respectively. This gave an overall system HRT of 3.05 and 1.8 days (including UASB, aerobic stage and settler). Figures 5.1 and 5.2 present a schematic diagram and a photograph respectively of the combined anaerobic-aerobic treatment system used in Experiments 2 and Expts 3.0-3.4.

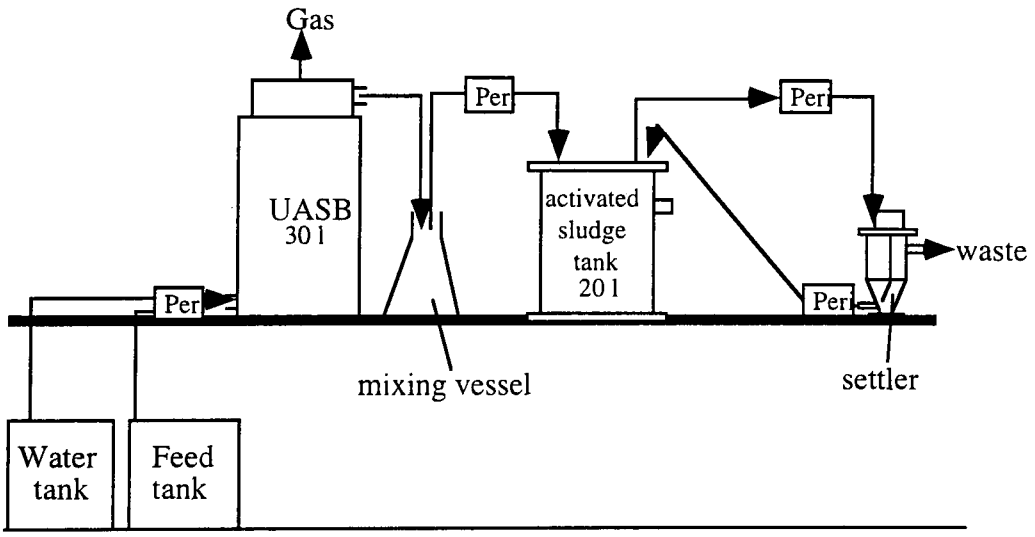


Figure 5.1 Schematic Lay-Out Of Combined Anaerobic-Aerobic Treatment System.

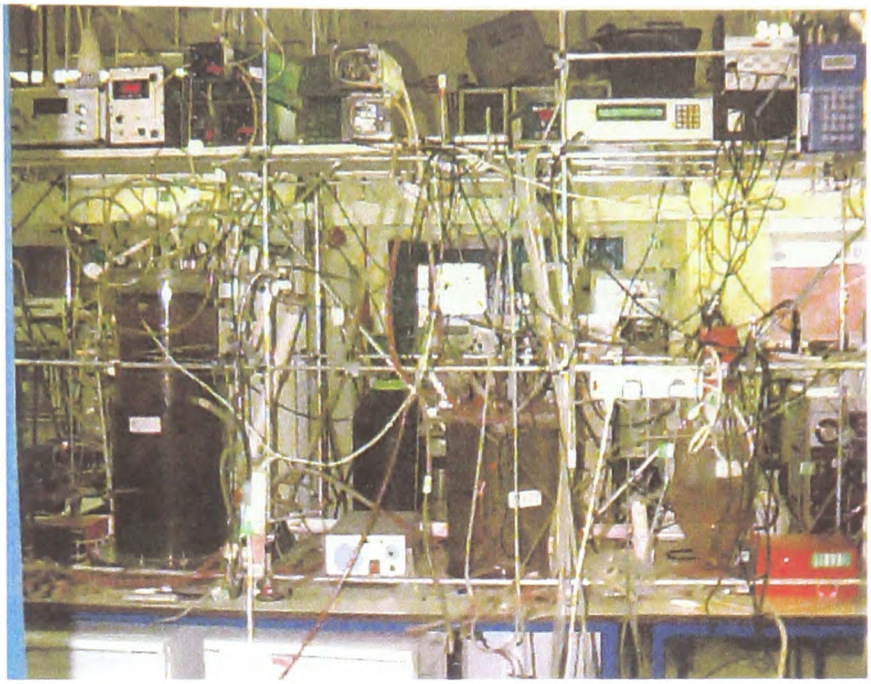


Figure 5.2 Photograph Of Combined Anaerobic-Aerobic Treatment System.

5.2.2 Analysis.

The COD, BOD and true colour of STE, UASB effluent and final effluent were measured. The quantity and composition of the biogas were also determined. In Experiment 2 samples for VFA concentration, bicarbonate alkalinity, and pH were extracted from a spare port at the top of the reactor (Figure 2.3). In Expts 3.1-3.4 samples for these measurements were removed from the recycle/sample port of the 30 l UASB. The TS and VS of granules were measured prior to filling the UASB and at the end of Experiment 3. Ion Exchange HPLC analysis of UASB effluent was carried out on samples from days 67 (Expt 3.3) and 74 (Expt 3.4) to determine whether nutrient carry-over from the anaerobic to the aerobic stage was sufficient to provide an adequate BOD:N:P ratio.

5.3 Results and Discussion.

5.3.1 TS and VS.

The granules added to the UASB gave a TS and VS of 60 (SD: 2.28; n: 6) and 49 (SD: 1.85; n: 6) g l^{-1} reactor respectively. This gave a lower concentration of biomass than that used in Experiment 1. Some wash-out of granules was observed over time and by the end of Experiment 3 the TS and VS were 33.7 and 26.1 g l^{-1} reactor, a loss of 44% and 47% respectively. This loss was similar to the 37 and 41% of TS and VS observed in the 5 l UASB (Section 4.3). Therefore loss of biomass remained a problem. The sludge and volumetric loading rates (B_x and B_v) can be seen in Table 5.2.

Table 5.2. Sludge Loading Rates (B_x) And Volumetric Loading Rates (B_v) in the UASB.

Experiment/ Expt	*Sludge loading rates (B_x: g COD g^{-1} VS d^{-1})	Volumetric loading rates (B_v: g COD l^{-1} reactor d^{-1})
2	0.053	1.39
3.0	0.041	1.06
3.1	0.102	2.67
3.2	0.063	1.66
3.3	0.105	2.74
3.4	0.069	1.81

*based on the biomass present at the end of the Experiment.

A range of B_x was examined in Experiments 2 and 3. The range of B_x used in the 5 l UASB (Section 4.3) fitted into this range. The maximum B_x values obtained for the 30 l UASB (Table 5.2) were similar to the 0.11-0.12 g COD g^{-1} VSS d^{-1} obtained in the ITD at a 2.8 d HRT (Section 4.3). However they were below the values achieved by An *et al.* (1996) of 0.269 g COD g^{-1} VSS d^{-1} and Zhu *et al.* (1994) of 0.75 g COD g^{-1} VSS d^{-1} for UASBs fed with dye wastes. Therefore this UASB was less heavily loaded than others used to treat textile wastes.

With the exception of Expt 3.0 the B_v values exceeded the 1.24 g COD l^{-1} d^{-1} obtained in the ITD at a 2.8 d HRT (Section 4.2.1). The B_vs achieved in the 5 l UASB at a 2 and

1.73 d HRT (Section 4.2.1), when the UASB operated without exhibiting signs of intolerance, were within the range achieved in Experiments 2 and 3 (Table 5.2). The B_v for Expts 3.1 and 3.3 approached the 2.8–3.12 g COD l^{-1} d^{-1} achieved when treating dye manufacturing wastes at a 10 hour HRT (estimated from Zhu *et al.*, 1994). However, they were below the 5.0 g COD l^{-1} d^{-1} recommended by An *et al.* (1996). The B_v values for Experiment 2 and Expts 3.0, 3.2 and 3.4 were below the reported and recommended values. Therefore in some Expts the volumetric loading rates were comparable with other systems although the sludge loading rates were lower.

5.3.2 Experiment 2 and Expt 3.0.

Table 5.3 presents the results obtained in Experiment 2 and Expt 3.0. The percentage reduction in parameters subsequent to anaerobic and aerobic treatment was calculated from the mean results as in Section 4.3.

The COD in Expt 3.0 was slightly lower than the projected COD of 2340 mg l^{-1} indicating that starch coagulation may have affected this Expt. The percentage COD removal was greater in Expt. 3.0 than in Experiment 2. This was due to the fact that the dye, which was not readily biodegradable, comprised 29% of the overall COD in Experiment 2 compared to only 4% in Expt 3.0 (Table 5.3). Therefore more of the COD in Expt 3.0 was attributable to the presence of starch and hence was readily removable, giving rise to COD removal superior to that achieved in Experiment 2. There was a statistically significant difference (99.9% CI) in the amount of COD removed anaerobically in Experiment 2 and Expt 3.0 (2183 and 1163 mg l^{-1} respectively). This difference was attributable to the longer HRT in Experiment 2. However, as the quantity removed in Experiment 2 was higher, dye concentration can be said not to inhibit anaerobic COD removal. The gas yield in Expt 3.0 was similar to the theoretical expected value of 0.35 l CH_4 g COD d^{-1} (Section 1.5.2.1). The gas yield was not measured in Experiment 2.

Table 5.3 Results Obtained After 3 Anaerobic HRT In (a) Experiment 2 and (b) Expt 3.0.

	(a) Experiment 2			(b) Expt 3.0		
Parameter	mean	SD	n	mean	SD	n
Starch:Dye Ratio	1.27			12.7		
Starch Contribution to STE COD (%)	53			72		
Dye Contribution to STE COD (%)	29			4		
BA (mg l^{-1} $CaCO_3$)	2707	(421)	7	1688	(206)	14
pH	8.0	0.2	6	7.4	0.12	6
COD STE (mg l^{-1})	*3473	(679)	118	1809	(267)	14
COD UASB effluent (mg l^{-1})	2550	(643)	8	646	(119)	14
COD final effluent (mg l^{-1})				392	(45)	5
Anaerobic COD removal (%)	27			64		
Aerobic COD removal (%)				14		
True colour STE (abs units)	7.99	(2.95)	6	0.84	(0.21)	13
True colour UASB effluent (abs units)	1.52	(0.53)	6	0.38	(0.06)	13
True colour final effluent (abs units)				0.34	(0.02)	3
Anaerobic colour removal (%)	81			55		
Aerobic colour removal (%)				5		
CH_4 (%)	75.3	(3.3)	6	71.3	(2.4)	14
Methane yield (l CH_4 g^{-1} COD removed)				0.33		

*as in Table 3.4.

The bicarbonate alkalinity was adequate in both cases, being in excess of the recommended minimum of 1000 mg l^{-1} as $CaCO_3$ (Section 1.5.2.1). The pH of 8.0 was slightly higher than that in Experiment 1, but by Expt 3.0 the pH had returned to the more typical value of 7.4.

A greater percentage reduction in true colour was achieved in Experiment 2 although the colour was not reduced to the same optical density as Expt 3.0. This may be attributable to the longer retention time of Experiment 2 and may also be partly attributable to adsorption. Expt 3.0 had a final colour removal of 60%, compared to the 81% removal achieved in Experiment 2 by anaerobic treatment alone, despite the higher dye concentration. However, the final colour in Experiment 2 was higher (Table 5.3) due to the high initial dye concentration (Table 5.1). Little colour was removed aerobically in Expt 3.0.

5.3.3 Step change Expts.

Some of the results obtained in Expts 3.1-3.4 can be seen in Table 5.4.

5.3.3.1 COD and BOD.

Most COD removal was achieved by the UASB in Expts 3.1-3.4 (Figure 5.3). The anaerobic COD removal ranged from 49-71% and therefore was higher than the 27-35% obtained in the UASB at 1.5 g l^{-1} dye (Tables 4.2 and 5.3). The increased COD removal confirmed that the dye was not very biodegradable. The measured CODs of Expts 3.1-3.4 were higher than the projected CODs by 14-21% (Table 5.4). However, due to the error associated with COD measurement the difference may not be significant. There was no significant difference in STE COD between Expts 3.1 and 3.3 (1.9 g l^{-1} starch; p: 0.5625), and between Expts 3.2 and 3.4 (0.95 g l^{-1} starch; p: 0.2908). Therefore Expts with the same starch concentrations had similar STE CODs at 0.075-0.15 g l^{-1} dye. Hence at this range of dye concentrations, the contribution of dye to COD was not significant. There was a significant difference (99.9% CI) in COD of UASB effluent between Expts 3.1 and 3.3, however, Expt 3.1 having the higher value. No significant difference was observed between Expts 3.2 and 3.4 (p: 0.6646). The dye concentration in Expt 3.1 was lower than in Expt 3.3, hence the difference was not attributable to inhibition or poor dye degradability. Therefore it appears that the problems experienced prior to Expt 3.1 (Section 5.2.1) affected the efficiency of the UASB in this Expt. This is despite the fact that the measurements were taken after three retention times had passed, and therefore the UASB could be expected to be in steady state. The poor performance of the UASB in Expt 3.1 resulted in a higher COD and BOD entering the activated sludge tank (Table 5.4). The similarity between UASB effluent COD in Expts 3.2 and 3.4 (0.95 g l^{-1} starch) indicates that at 0.075-0.15 g l^{-1} dye similar COD removal is obtained at the same starch concentration.

There was no significant difference between percentage aerobic COD removal achieved in Expts 3.2-3.4. However, in Expt 3.1 a high percentage of COD was removed aerobically

Table 5.4 Effects Of Step Changes Of Dye And Starch Loadings On System Performance After 3 Anaerobic HRT.

Parameter	Expt 3.1			Expt 3.2			Expt 3.3			Expt 3.4		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
Starch:Dye Ratio	25.3			12.7			12.7			6.33		
Starch Contribution to STE COD (%)	73			58			72			56		
Dye Contribution to STE COD (%)	2			3			4			6		
Projected COD (mg l^{-1})	2290			1450			2340			1500		
#COD STE (mg l^{-1})	2670	(127)	5	1655	(202)	5	2737	(216)	5	1813	(210)	4
#COD UASB effluent (mg l^{-1})	1358	(86)	3	695	(218)	3	802	(47.7)	3	615	(77)	2
#COD final effluent (mg l^{-1})	734	(120)	3	548	(118)	3	714	(54)	3	563	(7.8)	2
#BOD STE (mg l^{-1})	1136			1111			1743			1743		
#BOD UASB effluent (mg l^{-1})	698			290			360			343		
#BOD final effluent (mg l^{-1})	69			73			317			199		
COD:BOD STE	2.35			1.49			1.57			1.04		
COD:BOD UASB effluent	1.95			2.40			2.23			1.79		
COD:BOD final effluent	10.6			7.51			2.25			2.83		
TVFA UASB effluent (mg l^{-1})	798	(719)	3	264	(99)	2	238	(0.4)	2	152	(-)	1
CH_4 (%)	59.7	(3.1)		68.2	(1.9)	3	71.6	(2.2)	3	72.7	(1.9)	2
CH_4 yield (l CH_4 g^{-1} COD removed)	0.21			0.23			0.24			0.20		

mean (SD) n where applicable

#each n is the mean of three replicates

(23.4%), differing significantly from the 2.9-8.9% removed in Expts 3.2-3.4 (95-99% CI). Hence the aerobic stage compensated for the poorer UASB performance in Expt 3.1. There was a significant difference between the final effluent COD of Expts 3.3 and 3.4 (95% CI), Expt 3.3 having the higher value. However there was no significant difference in the final effluent COD of the other Expts. Therefore the combination of anaerobic and aerobic treatment often gives a final effluent with a similar COD despite variation in STE COD, and despite poor UASB performance. This consistency illustrates the usefulness of the aerobic stage subsequent to anaerobic treatment in polishing effluent.

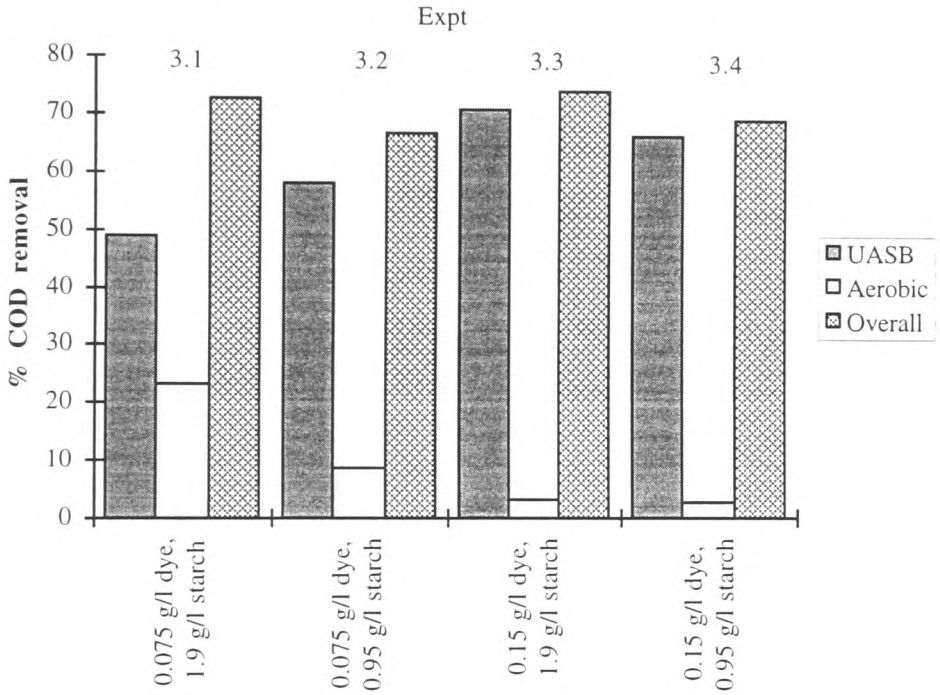


Figure 5.3 Percentage Of COD Removed By UASB, Aerobic Stage And Overall In Expts 3.1-3.4.

As with the COD, in most cases the majority of BOD was removed anaerobically. However, in Expt 3.1 aerobic BOD removal was higher than COD removal. Samples of STE, UASB effluent or final effluent with similar CODs did not exhibit similar BODs (Table 5.4). This can be attributed to the large error associated with BOD measurement. A greater aerobic BOD removal was achieved in Expt 3.1 than in Expts 3.2-3.4 (Figure 5.4) indicating once more that the aerobic stage was compensating for poor anaerobic performance. The COD:BOD ratio of the STE was ≤2.35 in all Expts 3.1-3.4 showing that the STE was biodegradable. The COD:BOD ratio increased during treatment in

Expts 3.2-3.4 although the increase after anaerobic digestion was small (Table 5.4). Substantial improvement in COD:BOD of UASB effluent by means of aerobic treatment was observed only in Expts 3.1 and 3.2, indicating that in later Expts the effectiveness of the aerobic stage was reduced. Some BOD remained at the end of the aerobic stage indicating that the STE was not fully degraded by means of combined treatment. However, it is possible that these BOD results were over-estimated by as much as 40% (Section 2.6.2).

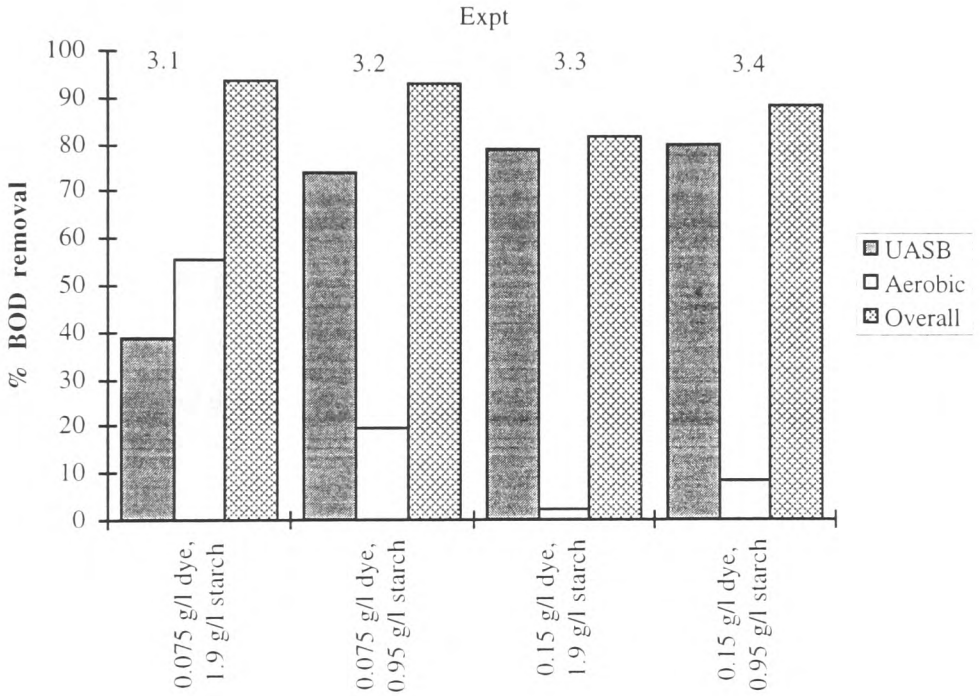


Figure 5.4 Percentage Of BOD Removed By UASB, Aerobic Stage And Overall In Expts 3.1-3.4.

With the exception of Expt 3.1, anaerobic COD removal was similar to the ~60% achieved by Zhu *et al.* (1994) and An *et al.* (1996) (Section 1.5.2.3). The aerobic COD removal for Expts 3.2-3.4 was lower than the 25-30% reported by Zhu *et al.* (1994) and the 23% reported by An *et al.* (1996) although these values were approached in Expt 3.1. The overall COD removal by means of combined treatment, ranging from 67-74%, was below the 83-90% achieved by Zhu *et al.* (1994) and An *et al.* (1996) but close to the 74-82% achieved by Zaoyan *et al.* (1992). The overall BOD removal in Expts 3.1 and 3.2

was similar to the 95% achieved by Zaoyan *et al.* (1992) while in Expts 3.3 and 3.4 it was lower.

5.3.3.2 Gas Measurements.

The highest rate of gas production was achieved in Expt 3.3 (646 ml l^{-1} reactor d^{-1}) with the lowest in Expt 3.2 and 3.4 (320-330 ml l^{-1} reactor d^{-1}). This showed that high STE CODs were associated with higher rates of gas production and vice versa. The methane yield for Expts 3.1-3.4 ranged from 0.20-0.24 l CH_4 g^{-1} COD removed. Therefore the yield was lower than the expected 0.35 l CH_4 g^{-1} COD removed (Section 1.5.2.1), for the same potential reasons as described in Section 4.3.2. The rates of gas production and methane yields g^{-1} COD removed exceeded those obtained for the ITD and 5 l UASB (Section 4.3.2). The biggest difference was in the rates of gas production, which were between 3 and 6 times greater than those obtained in Section 4.3.2. As the B_x and B_v used in Experiment 1 were within the range tested here, this indicated that much of the difference was attributable to different biodegradability of the STE used to feed the reactor.

5.3.3.3 Bicarbonate Alkalinity and Volatile Fatty Acids.

Figure 5.5 shows the concentration of TVFAs in the STE and UASB during Expts 3.1-3.4.

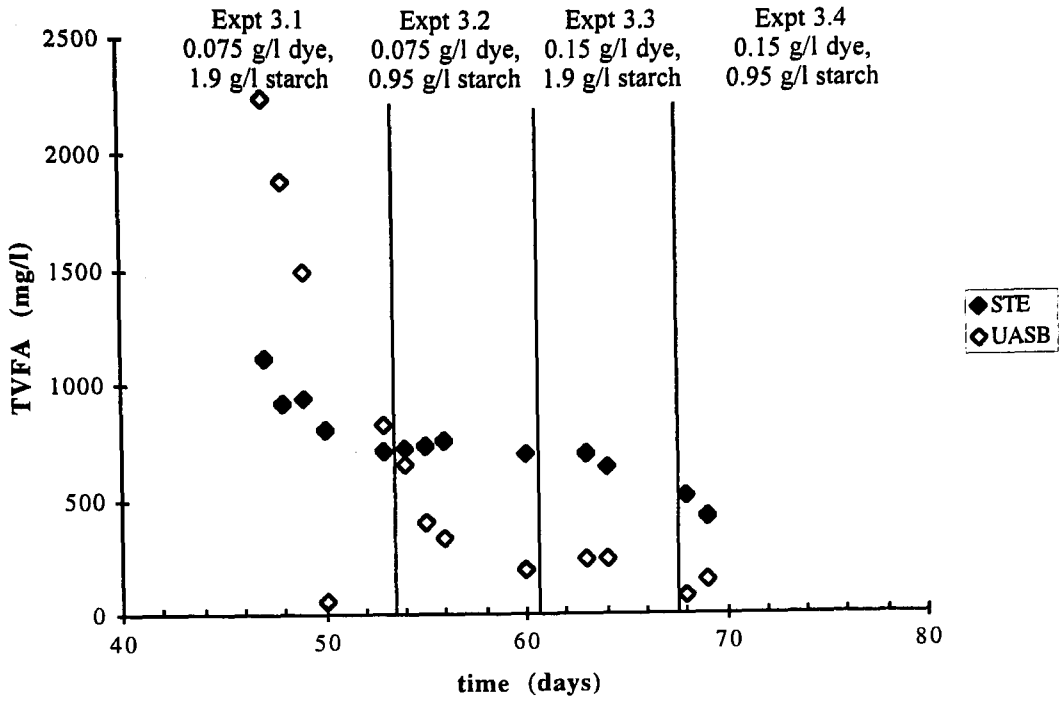


Figure 5.5 TVFA Concentration In The UASB In Expts 3.1-3.4.

It was seen that the TVFA concentration in the UASB was high at the start of Expt 3.1 and fell continuously until the end of Expt 3.2, remaining stable thereafter. The BA was measured on-line by a colleague during this period and was approximately 1500 mg l^{-1} $CaCO_3$ throughout. This gave a TVFA:BA for Expt 3.1 of 0.53, which was in excess of the 0.3 found in stable systems (Section 1.5.2.1). These results showed that the UASB was recovering from previous operational difficulties, confirming the cause of the poor COD removal in Expt 3.1 (Section 5.3.3.1). The low TVFA concentrations subsequent to Expt 3.1 showed that the UASB had recovered and was not stressed. The TVFA:BA ratios for Expts 3.2-3.4 were 0.1-0.18 and were therefore indicative of stable systems. Hence it was concluded that B_v of up to at least 2.7 g COD l^{-1} d^{-1} and B_x of up to 0.105 g COD g^{-1} VS d^{-1} (Table 5.2) could be tolerated by the UASB without difficulty. In Experiment 1 TVFAs increased at a B_v of 3.47 g COD l^{-1} d^{-1} (Figure 4.5), indicating a tolerance lying between 2.01-3.47 g COD l^{-1} d^{-1}. From the results obtained in Experiment 3 the limit could be narrowed down to 2.7-3.47 g COD l^{-1} d^{-1}. The B_v of 3.47 g COD l^{-1} d^{-1} in the 5 l UASB in Experiment 1 corresponded to a sludge loading rate of 0.087 g COD g^{-1} TS d^{-1}. The B_x achieved in Expts 3.1 and 3.3 exceeded this at

0.1 g COD g^{-1} VS d^{-1} although the B_v was lower, at 2.7 g COD l^{-1} d^{-1} (Table 5.2). This indicated that the UASB had become adapted to higher B_x.

5.3.3.4 Colour.

The results obtained from true colour measurement in Expts 3.1-3.4 are represented in Figure 5.6 and can also be seen in Table 5.5. The majority of the colour was removed in the anaerobic stage with a smaller percentage removed aerobically. This differed to the observations in Section 4.3.4 where there was a slight increase in colour in the activated sludge stage. Therefore it further indicates that the increase observed previously may have been due to the higher aerobic pH in Experiment 1. A higher percentage anaerobic true colour removal was observed at higher dye concentrations (Expts 3.3 and 3.4 compared to Expts 3.1 and 3.2). This corresponded to a higher rate of colour removal in terms of absorbance units removed per day. At higher dye concentrations colour was not reduced to the same optical density as the lower dye concentrations (Expts 3.1 and 3.2) after combined anaerobic-aerobic treatment (Table 5.5). Therefore the initial dye concentration was important in determining the final colour of treated effluent.

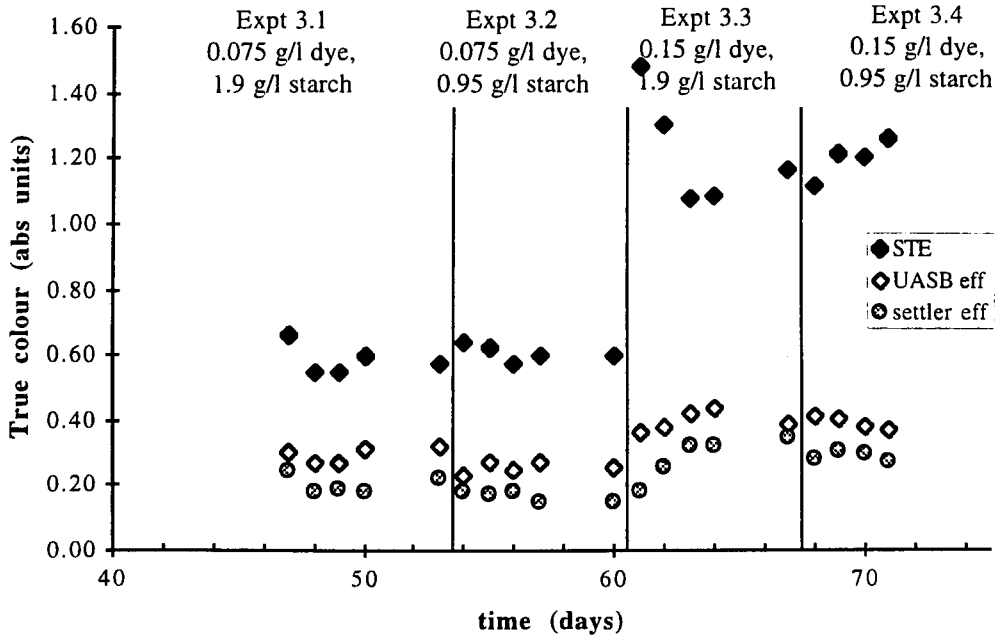


Figure 5.6 Colour Of STE, UASB Effluent And Final Effluent For Expts 3.1-3.4.

Table 5.5 Colour Results For Expts 3.1-3.4.

Parameter	Expt 3.1			Expt 3.2			Expt 3.3			Expt 3.4		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
Dye concentration (g l^{-1})	0.075			0.075			0.15			0.15		
True colour STE (abs units)	0.58	(0.05)	5	0.60	(0.03)	5	1.22	(0.17)	5	1.20	(0.06)	5
True colour UASB effluent (abs units)	0.30	(0.03)	3	0.25	(0.02)	3	0.41	(0.03)	3	0.37	(0.002)	2
True colour final effluent (abs units)	0.19	(0.02)	3	0.16	(0.02)	3	0.33	(0.01)	3	0.28	(0.01)	2
Anaerobic colour reduction (%)	48.3			58.3			66.4			69.2		
Aerobic colour reduction (%)	19.0			15.0			6.6			7.5		
Overall colour reduction (%)	67.2			73.3			73.0			76.7		

Figure 5.7 shows STE containing 0.075 g l^{-1} dye and 0.95 g l^{-1} starch prior to treatment, after anaerobic digestion and after combined anaerobic-aerobic treatment (Expt 3.2). This illustrates the anaerobic colour removal and the fact that little colour was removed by subsequent aerobic treatment. It therefore highlights the importance of the role that anaerobic treatment plays in removal of colour from textile effluent.

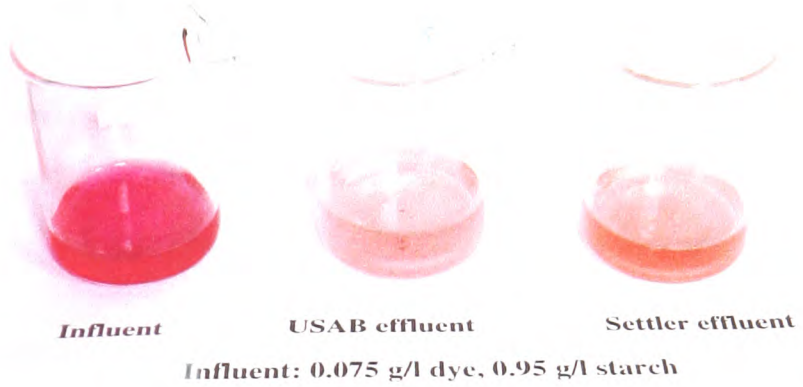


Figure 5.7 Photograph Of STE (Influent) Containing 0.075 g l^{-1} Dye And 0.95 g l^{-1} Starch; UASB Effluent; And Final (Settler) Effluent.

The anaerobic colour removal in Expt 3.1 was below the >50% reported by the manufacturers (Section 3.2.1). Expt 3.1 had the highest starch:dye ratio in Experiment 3 and therefore should have exhibited good colour removal. These results therefore further illustrate poor UASB performance in Expt 3.1. The anaerobic colour removal achieved in Expts 3.2-3.4 was in excess of this minimum and was also similar to, or in excess of, the 55-60% anaerobic colour removal achieved by Boe *et al.* (1993). However, it was below the ~80% anaerobic colour removal cited by An *et al.* (1996) and the 89 to >99% achieved by Razo-Flores *al.* (1997) when treating two azo dyes with a UASB. The aerobic colour removal in Expts 3.1 and 3.2 was in excess of the <10% cited by the dye manufacturers, Boe *et al.* (1993) and An *et al.* (1996), although that achieved for Expts 3.3 and 3.4 was within this range. The highest percentage aerobic colour removal corresponded with the highest aerobic COD and BOD removal. From the literature, colour removal in this stage is more likely to be attributable to adsorption than degradation (Section 1.5.2.2). The

colour removal from combined anaerobic-aerobic treatment was in excess of the 59% achieved by Loyd *et al.* (1992), similar to the 72% achieved by Zaoyan *et al.* (1992) but below the 90% removal achieved by An *et al.* (1996). Therefore anaerobic-aerobic treatment of this STE produced colour removal within the range of that achieved in published work. The final effluent was still coloured with a true colour value of 0.16-0.33 absorbance units. Therefore further treatment would be required.

There was no statistically significant difference in the colour of STE or UASB effluent in Expts with the same dye concentration (Expts 3.1 with 3.2 and Expts 3.3 with 3.4; p: 0.0603-0.3560). The starch:dye ratios in Expts 3.1 and 3.3 were twice those of Expts 3.2 and 3.4 respectively (Table 5.4). As the colour of the UASB effluent was similar for Expts with the same dye concentration but different starch:dye ratios (Expts 3.1 and 3.2; Expts 3.3 and 3.4), it can be said that higher starch:dye ratios did not enhance colour removal at the dye and starch concentrations tested here. This indicated that either sufficient starch was present for good dye decolourisation, or a greater excess of starch was required. Although there was no significant difference in colour of STE or UASB effluent between Expts 3.1 and 3.2, the slight, non-significant differences in colour (Table 5.5) gave rise to a statistically significant difference in anaerobic colour removal between the Expts (95% CI).

Colour is affected by pH and therefore should ideally be measured at a constant pH, preferably pH 7. The colour measurements here were taken in preparation for putting colour measurements on-line. Therefore samples could not be pH adjusted prior to measurement. The pH of the anaerobic effluent was approximately 7 when samples were analysed immediately after collection. The pH of final effluent was also approximately 7 due to the pH control system used with the activated sludge stage. Therefore it was only the STE pH that was in excess of neutral. Tests were done on STE containing different combinations of starch and dye to determine the effect of pH on colour measurement. It was found that between pH 7.0 and 10.7 colour variation of STE containing 1.9-3.8 g l^{-1} starch and 0.15-0.75 g l^{-1} dye was less than or equal to 6%. Almost all the STE samples were within this pH range, with the occasional exception in STE containing 3.8 g l^{-1} starch (days 22-123, Experiment 4) when the pH was, on occasion, observed to rise as high as

11.3. Therefore it was concluded that measurement of samples without pH adjustment usually produced satisfactory results.

5.3.4 Aerobic Stage.

The MLSS in the activated sludge stage declined over the experimental period (Figure 5.8). The daily MLSS of Expts 3.1-3.4 was examined (Table 5.6) for all days of each Expt. The biggest decrease was seen in Expt 3.1 as evidenced by the high standard deviation of the measurements. The pH adjustment of the aerobic stage was successful and maintained the activated sludge stage at pH 7. Therefore pH was not the cause of loss of biomass. Anaerobic conditions at the bottom of the settling tank can result in death of biomass and hence loss of solids from the system when aerobic solids are not recycled continuously. Due to the continual loss of biomass the aerobic stage can not be said to reach steady state.

Table 5.6 Mixed Liquor Suspended Solids Concentration And F:M To the Aerobic Stage In Expts 3.1-3.4.

	MLSS ($g\ l^{-1}$)			F:M
Expt	mean	SD	n	($g\ BOD\ g^{-1}\ MLSS\ d^{-1}$)
3.1	2.26	(0.7)	5	0.46
3.2	1.35	(0.2)	5	0.32
3.3	1.21	(0.22)	5	0.45
3.4	0.98	(0.14)	4	0.53

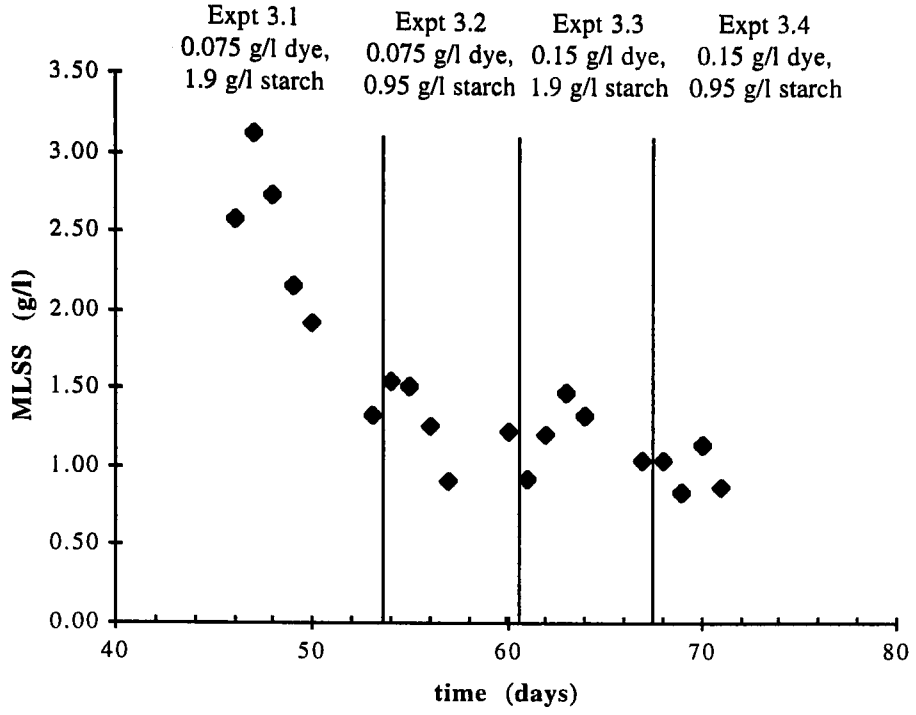


Figure 5.8 MLSS vs Time For Expts 3.1-3.4.

The concentration of nitrogen and phosphorus entering the aerobic stage was measured to determine whether sufficient quantities were present to provide adequate BOD:N:P ratios. A mean concentration of 106 mg N l^{-1} was detected in UASB effluent from the ammonium detected. The phosphorus concentration was calculated to be 66 and 17.6 mg l^{-1} (days 67 (Expt 3.3) and 74 (Expt 3.4) respectively) from the phosphate detected by means of HPLC Ion exchange chromatography. Comparison of these results with those obtained from STE analysis (Section 3.4.3) showed some removal of nitrogen occurred during anaerobic treatment. The phosphorus concentration present on day 67 was higher than that detected in STE (Section 3.4.3) while that on day 74 showed some removal of this element. These results gave a mean BOD:N:P of 5.5:1.6:1 for Expt 3.3, and 19:6:1 for Expt 3.4. When these ratios were compared with the recommended 100:5:1 (Section 1.5.2.2) it was seen that the nutrient concentrations were in excess of those recommended for activated sludge systems, even at the lowest phosphorus concentration. Therefore sufficient nutrients were present to permit good operation of the aerobic stage.

The F:M ratios were found to vary from 0.32 to 0.53 g BOD g^{-1} MLSS d^{-1} in Expts 3.1-3.4. The values for Expts 3.1-3.3 were within the range cited in Section 1.5.2.2 while that for Expt 3.4 was slightly in excess (Table 5.6). At F:M in excess of 0.3 bulking would normally be expected. No bulking was observed in this Expt, however, indicating that the activated sludge tank was not acting as would normally be expected. This may be attributable to the fact that sludge loading becomes higher as solids decrease. Given the COD, BOD and colour remaining in the final effluent, it can be seen that further treatment would be required prior to discharge.

The colour measurements obtained in Expt 3.2 were compared with results obtained from days 1-22, Expt 3.5 when an activated sludge tank was operated on STE (0.075 g l^{-1} dye, 0.95 g l^{-1} starch) that had not been anaerobically pre-treated. The MLSS during this period was 2.7 g l^{-1} (SD: 0.56; n: 15). The colour of STE was 0.52 (SD: 0.06; n: 7) and 0.56 absorbance units (SD: 0.13; n: 7) before and after aerobic treatment respectively. The difference in measurements was not statistically significant (p: 0.4461). It was concluded from these results that no colour removal was obtained by activated sludge treatment alone. By comparison, 73.3% colour removal was achieved in Expt 3.2 by means of combined anaerobic-aerobic treatment (Table 5.5). The COD before and after aerobic treatment was 1332 (SD: 461; n: 14) and 369 (SD: 118; n: 14) mg l^{-1} respectively. The percentage COD removal (72%) was therefore similar to the 67% achieved by combined anaerobic-aerobic treatment in Expt 3.2 (Table 5.4; Figure 5.3). Hence it can be concluded that aerobic treatment removed a similar quantity of COD to combined anaerobic-aerobic treatment but could not remove colour. Expt 3.5 therefore confirmed that colour removal cannot be achieved by aerobic treatment alone, and that anaerobic treatment can be used as a pre-treatment to remove colour. This concurred with Loyd *et al*'s. (1992) (Section 1.5.2.2) findings regarding colour. The findings of Expt 3.5 regarding biodegradation differed from those of Loyd *et al.* (1992) as a significant amount of COD was removed by anaerobic treatment alone (58%). However the COD removal was lower than that achieved by aerobic treatment alone.

5.4 Conclusions.

The anaerobic sludge loading rates (B_x) achieved were below the values achieved by other authors while the volumetric loading rates (B_v) in Expts 3.1 and 3.3 approached some reported loading rates. The UASB could operate at a B_v of up to at least 2.7 g COD l^{-1} d^{-1} without becoming stressed. Therefore the maximum B_v lay between 2.7 and 3.47 g COD l^{-1} d^{-1}.

The percentage anaerobic COD removal increased when the dye concentration was reduced from 1.5 g l^{-1} to 0.15 g l^{-1}. This confirmed that the dye exhibited poor biodegradability. However, in Expts 3.1-3.4 decreasing dye concentration from 0.15 g l^{-1} to 0.075 g l^{-1} had no significant effect on anaerobic COD removal. This indicated that at this concentration the dye contribution to STE COD was sufficiently low to have little impact on the STE biodegradability.

The majority of colour was removed in the anaerobic stage with a smaller percentage removed aerobically. A higher percentage true colour removal was achieved at higher dye concentrations (1.5>0.15>0.075 g l^{-1}) but the colour was not reduced to the same optical density as the lower dye concentrations. The overall colour removal was within the range of that achieved by other authors. The starch:dye ratio did not appear to affect colour removal at the dye and starch concentrations tested here (0.15 and 0.075 g l^{-1} dye; 1.9 and 0.95 g l^{-1} starch). This indicated either that sufficient starch was present for optimum dye decolourisation, or that a greater excess of starch was required.

The UASB was not working well in Expt 3.1 due to problems experienced previously. Despite this there was no significant difference in the COD of final effluent in Expts 3.1-3.4. Therefore the role of the aerobic stage in removing excess COD was proved. The role of the anaerobic stage in colour removal from textile effluents was also proved.

The adjustment of the aerobic reactor to pH 7 was successful but the aerobic biomass decreased throughout Expts 3.1-3.4. Nutrient analysis indicated that the quantities of nitrogen and phosphorus present were sufficient. The F:M ratios were in excess of the

recommended, yet no bulking was observed. It was therefore apparent that the aerobic stage was not working efficiently during the 33 days of continuous operation.

CHAPTER SIX - ANAEROBIC-AEROBIC TREATMENT OF SIMULATED TEXTILE EFFLUENT CONTAINING VARIED RATIOS OF STARCH AND DYE.

6.1 Introduction.

In Experiment 4 a 30 l UASB and a 20 l aerobic reactor were used, as in Experiment 3 (Figures 5.1 and 5.2). The tolerance of the UASB for different volumetric loading rates was investigated further in this Experiment, which is also described in O'Neill *et al.*, 1999d. The performance of the UASB and activated sludge tank at different loading rates were also assessed. The ratio of starch to dye was varied further in this Experiment and the ability of the treatment system to return to its initial performance after step changes of 1 week duration was examined. This involved seven step changes over a five month period. The effect of different concentrations of electron donor (starch) and electron acceptor (dye) on colour removal was examined. Analyses were carried out to determine whether aromatic amines were produced during anaerobic treatment of the STE. It is important to demonstrate that they are generated as this shows that decolourisation does not occur by means of adsorption only. Also it is important that these amines are degraded aerobically due to their reported toxicity (Section 1.5.2.1.1). Therefore the fate of such amines was also investigated.

6.2 Methods.

6.2.1 Reactors and Experimental Design.

The set-up was similar to that used in Expts 3.1-3.4 with the exception that a 10-fold concentrate of STE was used in Expts 4.0-4.4 and a x15 concentrate in Expt 4.5. The UASB was unfed at room temperature for 52 days prior to commencing this Experiment. Feeding of the UASB at a 1 d HRT commenced on day 1 and the activated sludge tank was operated from day 8 with new sludge. The DO of the 20 l aerobic stage was controlled throughout Experiment 4 (Section 2.2.3) and the aerobic pH was controlled to

pH 7, as in Experiment 3. Solids were recycled from the settler continuously in order to reduce exposure of aerobic biomass to anaerobic conditions in the settler. There was a 20 day break in UASB operation between days 51 and 52 following which the aerobic stage was restarted on day 57. The UASB was then operated continuously until day 166, the period from days 123-166 being used for neural network testing, which is not reported here. After a 26 day break in operation the UASB during which time the UASB was at room temperature, unfed, feeding recommenced from days 167 to 186. The aerobic stage was refilled on day 171. The system was operated from days 186-228 under the same experimental conditions as days 167-186 in order to facilitate tests on the neural network, which are not reported here, and to collect samples to send away for analysis (Section 6.2.2). The overall system HRT was 1.8 days (UASB, aerobic stage and settler).

The rig was initially operated on a feed of 0.15 g l^{-1} dye and 1.9 g l^{-1} starch for 22 days (Expt 4.0). A programme of varying STE starch and dye concentrations was then followed (Table 6.1; Figure 6.1), testing higher concentrations of both substances than tested in Experiment 3. The system was operated for approximately 7 days in Expts 4.2-4.4, each followed by a return for 7 days to Expt 4.1, which had the same dye and starch concentrations as Expt 4.0 (Table 6.1). Each Expt 4.2-4.4 was repeated, thus Expt 4.1 spanned seven separate 1-week periods and monitored the ability of the reactor to return to the conditions of Expt 4.0 after Expts 4.2, 4.3 and 4.4. In Expts 4.3 and 4.4 the starch was doubled to 3.8 g l^{-1} and in Expts 4.2 and 4.4 the dye was increased 5-fold to 0.75 g l^{-1}. Expt 4.5 was performed using 0.45 g l^{-1} dye and 2.9 g l^{-1} starch. The results obtained in days 22-29 (Expt 4.3) were discarded due to problems in mixing the STE. These problems arose due to the high concentrations of starch in this Expt. A centrifugal pump was used from day 36 to keep the STE completely mixed and thus prevent a reoccurrence of this problem. In Expt 4.5 a concentrated supplement of OECD synthetic sewage (Section 2.2.3) was fed to the activated sludge tank at a rate of 1.4 litres per day. This attempted to imitate a textile effluent treatment plant where almost one third of the COD to the aerobic stage came from domestic sewage. Most textile effluent is treated in conjunction with domestic sewage (Section 1.5).

Table 6.1 Dye And Starch Concentrations, Their Ratio, Contribution To The Projected COD, And Loading Rates To UASB For Expts 4.0-4.5.

Expt	Days	Dye ($g\ l^{-1}$)	Starch ($g\ l^{-1}$)	Starch:Dye Ratio	Starch Cntb. (%)	Dye Cntb. (%)	*B_x ($g\ COD\ g^{-1}\ VS\ d^{-1}$)	B_v ($g\ COD\ l^{-1}\ d^{-1}$)
4.0	1-22	0.15	1.9	12.7	72	4	0.088	2.29
4.1	29-36; 44-51; 52-59; 66-73; 80-87; 95-101; 108-115	0.15	1.9	12.7	72	4	0.085	2.22
4.2	36-44; 73-80	0.75	1.9	2.53	62	17	0.104	2.71
4.3	22-29; 59-66; 87-95	0.15	3.8	25.3	84	2	0.143	3.73
4.4	101-108; 115-123	0.75	3.8	5.07	77	10	0.15	3.90
4.5	171-186	0.45	2.9	6.44	75	8	0.12	3.14

*based on biomass present at the beginning of the Experiment
B_x - sludge loading rates. B_v - volumetric loading rates
Cntb. - Contribution to projected COD

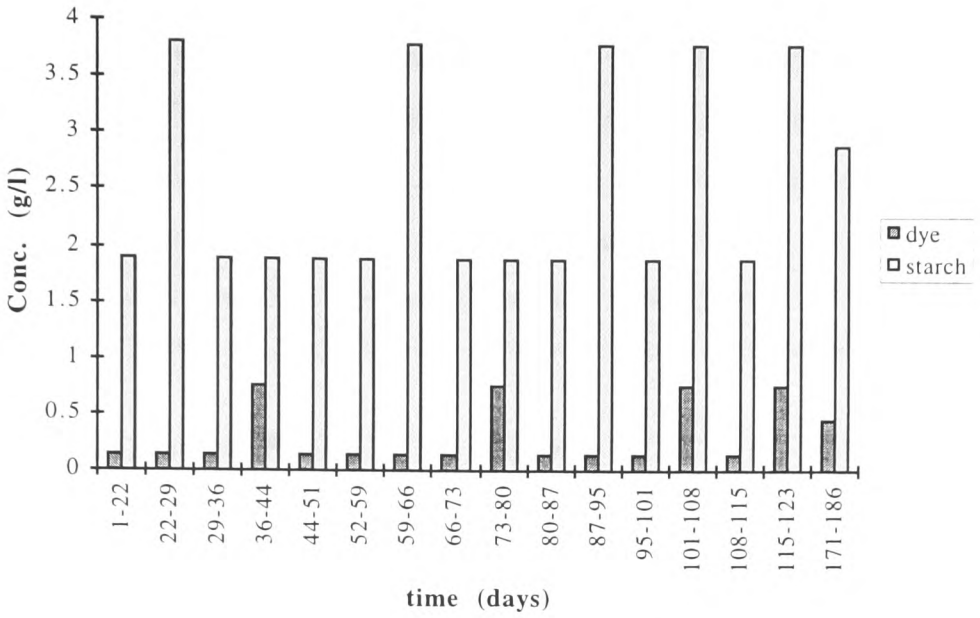


Figure 6.1 Dye And Starch Concentration (Conc.) In STE Feed vs Time in Experiment 4.

6.2.2 Analysis.

The solids content of the UASB at the beginning of Experiment 4 was that present at the end of Experiment 3 (Section 5.3.1) and was remeasured after Experiment 4 was complete. The BOD and COD of STE, UASB effluent and final effluent were measured. The VFA concentration, bicarbonate alkalinity, and internal pH of effluent extracted from the recycle/sample port of the 30 l UASB were also determined. The pH of the STE, UASB effluent and final effluent was measured off-line daily prior to determination of the true colour of samples. The percentage carbon dioxide in the biogas was measured on-line by a colleague and the percentage hydrogen sulphide present in the biogas was also determined. The MLSS of the aerobic stage was determined daily. The results in each repeat of Expts 4.1-4.4 were taken after 3 HRT to the anaerobic stage. The mean results were calculated from the average of each repeat (Table 6.1; Figure 6.1). Each 'n' in these Expts is therefore the number of repeats for each Expt. In Expts 4.0 and 4.5 each 'n' is the number of daily readings. The daily BOD and COD measurements were the mean of 3 replicates.

Samples of STE, UASB effluent and final effluent were sent for respiration inhibition testing on day 209 to Alcontrol Laboratories (Yorkshire, UK). HPLC-UV and Total Organic Nitrogen analyses were carried out (by IRSA, Italy) on samples taken on days 209 and 217. These attempted to determine qualitatively whether UV-adsorbing (i.e. aromatic) amino-derivatives were formed and/or degraded during each treatment step. Samples for both tests were taken under the conditions of Expt 4.5 although measurements for this Expt were not taken during this period.

6.3 Results and Discussion.

Results obtained from Experiment 4 are also presented in O'Neill *et al.* (1999d).

6.3.1 TS and VS.

At the start of Experiment 4 the UASB contained 33.7 g TS and 26.1 g VS l^{-1} reactor (Section 5.3.1). At the end of Experiment 4, 13.4 and 10.2 g l^{-1} reactor of TS and VS respectively were present, a decrease of 60-61% for both. The latter values were not truly representative of the biomass present during Experiment 4 as subsequent to Experiment 4, and prior to measurement of the biomass, large quantities of granules floated and were consequently lost from the UASB. Hence the sludge loading rates (Table 6.1) were calculated from the VS present at the beginning of the Experiment rather than at the end. The days of the Expts and their associated volumetric and sludge loading rates can be seen in Table 6.1.

The maximum sludge loading rate (B_x) obtained in the UASB was 0.15 g COD g^{-1} VS d^{-1} (Table 6.1). As in Experiment 3 (Table 5.2), this was below the B_x achieved by Zhu *et al.* (1994) and An *et al.* (1996) (Section 5.3.1). In Expts 4.0-4.5 the F:M in the UASB ranged from 0.041-0.098 g BOD g^{-1} VS d^{-1}. This was below the normal F:M in anaerobic systems (Section 1.5.2.1.2). However, as in Expts 3.1 and 3.3, the B_vs achieved in Expts 4.2 and 4.5 were within the range of that achieved by Zhu *et al.* (1994). The B_vs of Expts 4.3 and 4.4 exceeded this reported range, while the B_vs achieved in Expts 4.0 and 4.1 were

lower. All B_vs were below that recommended by An *et al.* (1996). Therefore it can be concluded that while the volumetric loading rates were within the range achieved by other authors, the sludge loading rates were below reported B_x values (Section 5.3.1).

6.3.2 Expts 4.0 and 4.1.

6.3.2.1 COD, BOD And Methane Yield.

The results of Expt 4.0 and Expt 4.1, which had the same STE composition (1.9 g l^{-1} starch, 0.15 g l^{-1} dye; Table 6.1), were compared to ascertain whether the Expt 4.0 performance conditions were regained between Expts 4.2, 4.3 and 4.4. There was no significant difference in COD of the STE, UASB effluent or final effluent between Expts 4.0 and 4.1 (p: 0.1256-0.7114). The percentage reduction in COD by each stage (after 3 HRT) was also similar, at 62-66% for the UASB and 14-17% for the aerobic stage. This gave an overall reduction of 79.1 and 80.2% for Expts 4.0 and 4.1 respectively (Table 6.2). Therefore the anaerobic and aerobic stages removed similar quantities of COD in both Expts.

The BOD results for Expt 4.1 were higher than those obtained for Expt 4.0. Given the similarity in COD results, this is probably attributable to the high error associated with this measurement. As seen with the COD results, most BOD was removed anaerobically. The overall percentage removal in BOD by each stage was similar, with 58-65% removal by the UASB and a further 34-37% removal after aerobic treatment (Table 6.2). This gave a total BOD removal of 96-99%. However, because there was only one BOD measurement for Expt 4.0 statistical comparison of the two Expts could not be made in relation to this parameter. Methane yields were 0.29 and 0.27 l CH_4 g^{-1} COD removed in Expts 4.0 and 4.1 respectively and were therefore also similar.

Table 6.2 COD And BOD In STE, UASB Effluent and Final Effluent For Expts 4.0-4.5.

Expt	STE (mg l^{-1})			UASB Effluent (mg l^{-1})			Final Effluent (mg l^{-1})		
	mean	SD	n	mean	SD	n	mean	SD	n
4.0 (COD)	2287	(285)	12	877	(130)	12	477	(92)	9
4.0 (BOD)	1068	-	1	377	-	1	14	-	1
4.0 (COD:BOD)	2.14			2.33			34.1		
4.1 (COD)	2222	(132)	7	750	(218)	7	441	(269)	7
4.1 (BOD)	1483	(162)	6	619	(131)	6	65	(35)	6
4.1 (COD:BOD)	1.50			1.21			6.78		
4.2 (COD)	2713	(131)	2	1303	(153)	2	911	(245)	2
4.2 (BOD)	1397	(49)	2	599	(130)	2	71	(12)	2
4.2 (COD:BOD)	1.94			2.18			12.8		
4.3 (COD)	3731	(152)	2	1462	(229)	2	444	(16)	2
4.3 (BOD)	2002	(16)	2	1151	(348)	2	102	(13)	2
4.3 (COD:BOD)	1.86			1.27			4.4		
4.4 (COD)	3902	(83)	2	1579	(49)	2	812	(139)	2
4.4 (BOD)	2550	(281)	2	1225	(75)	2	157	(10)	2
4.4 (COD:BOD)	1.53			1.29			5.2		
4.5 (COD)	3137	(341)	12	1205	(333)	12	723	(104)	11
4.5 (BOD)	1853	(336)	3	673	(134)	2	67	(9)	3
4.5 (COD:BOD)	1.69			1.79			10.8		

6.3.2.2 Bicarbonate Alkalinity and Volatile Fatty Acids.

The TVFA concentration in the UASB was similar (p: 0.2489) in Expts 4.0 and 4.1 (Table 6.3), most of the VFA being acetic acid. The pH of the UASB was 7.3 in both Expts (SD: 0.14; n: 13 and SD: 0.06; n: 7 for Expts 4.0 and 4.1 respectively). The BA was also similar (p: 0.3169) at 1766 (SD: 95; n: 13) and 1812 (SD: 91; n: 7) mg $CaCO_3$ l^{-1} in Expts 4.0 and 4.1. As the BA was similar and the TVFA concentrations were low in the two Expts, the UASB was not stressed or showing any signs of instability. Hence it can be said that the step change Expts 4.2-4.4 did not affect the performance of the UASB when operated under the conditions of Expt 4.0.

Table 6.3 VFAs In The UASB For Expts 4.0-4.5.

Expt	Acetic acid ($mg\ l^{-1}$)			Propionic acid ($mg\ l^{-1}$)			TVFA ($mg\ l^{-1}$)		
	mean	SD	n	mean	SD	n	mean	SD	n
4.0	339	(61)	13	44	(9.8)	13	388	(71)	13
4.1	275	(130)	7	45	(23)	7	328	(156)	7
4.2	206	(73)	2	25	(6.6)	2	236	(69)	2
4.3	759	(388)	2	177	(77)	2	963	(485)	2
4.4	593	(458)	2	118	(79)	2	728	(552)	2
4.5	268	(223)	12	83	(59)	12	373	(246)	12

6.3.2.3 Colour.

Most colour removal occurred anaerobically (Table 6.4). Anaerobic and overall true colour removal was better in Expt 4.0 than in Expt 4.1 (99.9% CI and 99% CI respectively). This could be due to adsorption occurring in the UASB in Expt 4.0. Although the UASB had been operated on this combination of dye and starch previously (Expts 3.0 and 3.3), the pause in operation prior to Experiment 4 gave the potential for a new dye equilibrium to be attained. A similar quantity of colour (14-15%) was removed aerobically in both Expts.

Table 6.4 True Colour At Each Stage And Percentage Overall Colour Removal By Means Of Combined Anaerobic-Aerobic Treatment For Expts 4.0-4.5.

Expt	True colour STE (abs units)			True colour UASB (abs units)			True colour settler (abs units)			Overall removal (%)
	mean	SD	n	mean	SD	n	mean	SD	n	mean
4.0	1.01	(0.07)	12	0.42	(0.07)	12	0.28	(0.08)	9	72.3
4.1	0.93	(0.07)	7	0.55	(0.07)	7	0.41	(0.09)	7	55.9
4.2	5.05	(0.11)	2	3.12	(0.26)	2	2.76	(0.44)	2	45.3
4.3	0.92	(0.04)	2	0.38	(0.01)	2	0.21	(0.01)	2	77.2
4.4	4.32	(0.13)	2	1.85	(0.12)	2	1.37	(0.01)	2	68.3
4.5	2.38	(0.33)	9	1.06	(0.11)	9	0.77	(0.24)	9	67.6

6.3.2.4 Aerobic Stage.

Table 6.5 illustrates the MLSS and F:M for the aerobic stage in Expts 4.1-4.5. The mean MLSS was 3.3 g l^{-1} (SD: 1.2; n: 11) in Expt 4.0 compared to 1.9 g l^{-1} (SD: 1.5; n: 7) in Expt 4.1. The change in MLSS over time can be seen in Figure 6.2.

Table 6.5 MLSS And F:M Obtained In Expts 4.0-4.5.

Expt	MLSS (g l^{-1})			F:M
	mean	SD	n	(g BOD g^{-1} MLSS d^{-1})
4.0	3.3	(1.2)	11	0.17
4.1	1.91	(1.5)	7	0.49
4.2	1.53	(0.2)	2	0.59
4.3	2.74	(2.1)	2	0.63
4.4	1.34	(0.03)	2	1.37
4.5	2.43	(0.92)	12	0.42

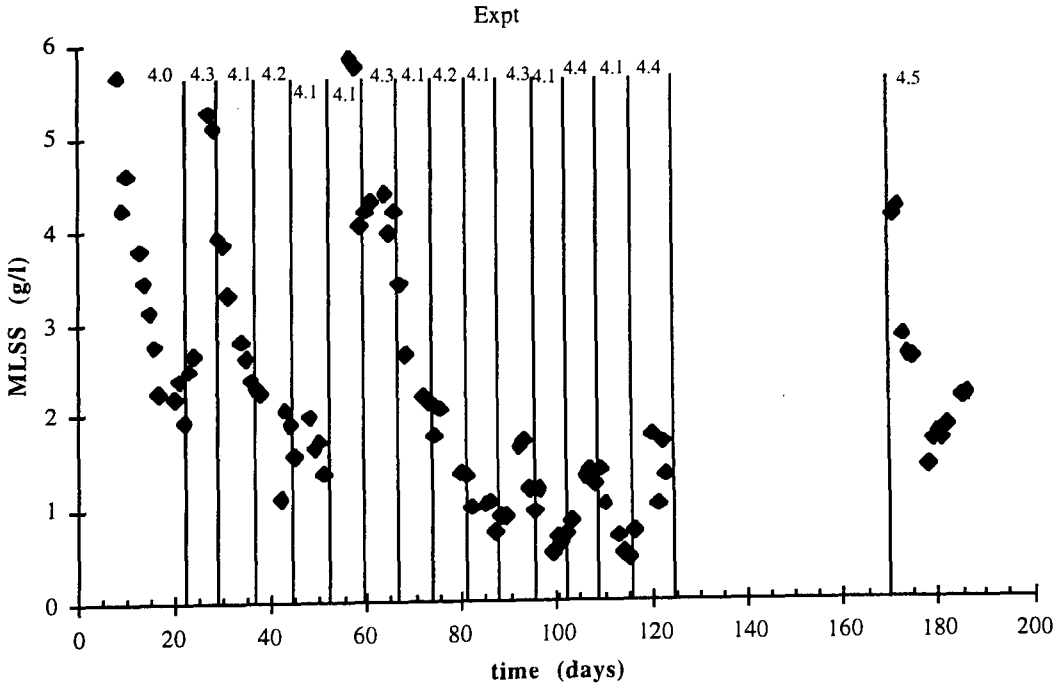


Figure 6.2 MLSS vs Time For Expts 4.0-4.5.

Biomass was lost throughout Expts 4.1-4.4. Therefore the difference in MLSS between Expts 4.0 and 4.1 was attributable to loss of biomass over time and hence does not reflect

on differences in response to the Expts. The F:M for Expt 4.0 (Table 6.5) was within the normal range found in aerobic systems (Section 1.5.2.2) while, due to the loss of MLSS, that in Expt 4.1 exceeded the normal range. However, these differences cannot be attributed to changed response to the STE caused by the step change experiments.

6.3.2.5 Summary.

With the exceptions of MLSS and colour removal, which were thought not to reflect on different response to operation, there was essentially no difference between response of combined anaerobic-aerobic treatment to Expts 4.0 and 4.1. This showed that the anaerobic stage could tolerate step changes in starch and dye concentration without any change in effectiveness of operation. Therefore differences observed in Expts 4.2-4.5 were due to changes in experimental conditions and not due to underlying changes in reactor microbiology.

6.3.3 Expts 4.1-4.5.

6.3.3.1 COD and BOD.

The percentage COD removal from each treatment stage for Expts 4.1-4.5 after 3 HRT can be seen in Figure 6.3. As seen previously (Sections 4.3.1 and 5.3.3.1), most removal occurred in the UASB. At 3.8 g l^{-1} starch (Expts 4.3 and 4.4) similar anaerobic COD removal (60-61%) was achieved at both dye concentrations. However, at 1.9 g l^{-1} starch the higher dye concentration (0.75 g l^{-1}) gave poorer COD removal (52% compared to 66%). In Expts 4.3 and 4.4 the aerobic stage performed a greater role in COD removal, at 27 and 20% compared to 14-15% for Expts 4.1, 4.2 and 4.5. It was seen from Table 6.2 that a higher COD exited from the UASB in Expts 4.3 and 4.4. The high BOD of this effluent showed that much of it was biodegradable (Table 6.2).

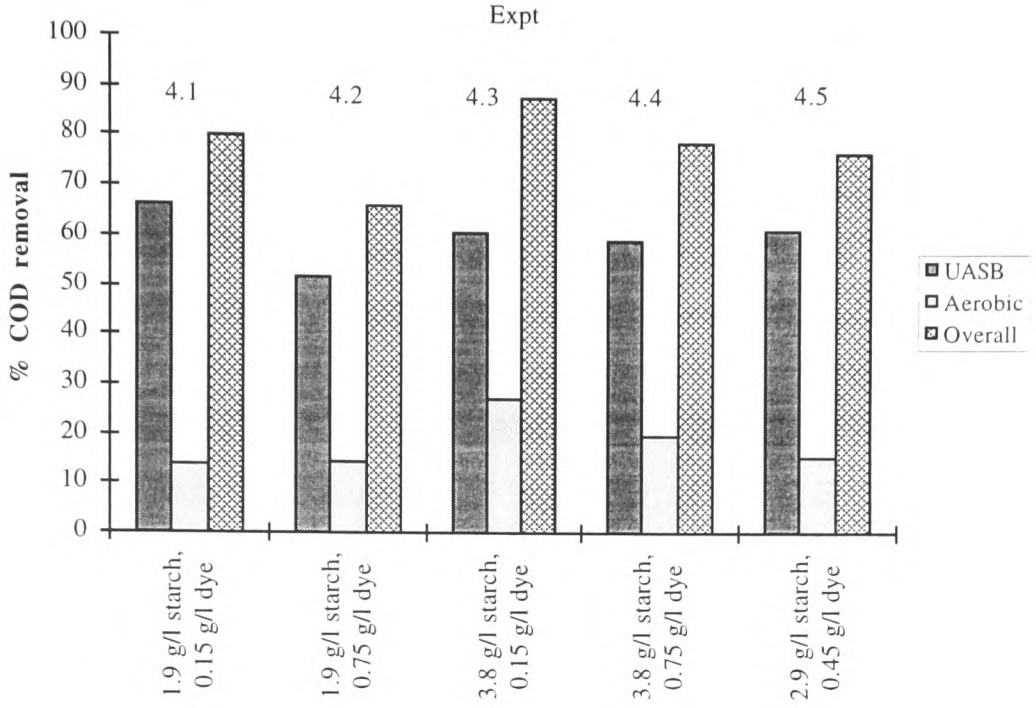


Figure 6.3 Percentage COD Removal By UASB, Aerobic Stage And Overall For Each Expt.

The COD of the final effluent was similar for both starch concentrations at the same dye concentration (p: 0.9898 for Expts 4.1 and 4.3; p: 0.6659 for Expts 4.2 and 4.4). The lowest final effluent COD (~440 mg l^{-1}) was obtained at 0.15 g l^{-1} dye at both 1.9 and 3.8 g l^{-1} starch (Expts 4.1 and 4.3; Table 6.2). In these Expts the dye contribution to the projected COD was lower than the other Expts at 2-4%, compared to 8-17% in Expts 4.2, 4.4 and 4.5 (Table 6.1). The poorest overall COD reduction (66%) was obtained in Expt 4.2 (1.9 g l^{-1} starch and 0.75 g l^{-1} dye). This was attributable to the fact that the dye comprised 17% of the projected COD in this Expt (Table 6.1), and thus confirmed its poor biodegradability (Section 3.2.1). The COD of the final effluent in Expt 4.5 was between the values obtained at 0.15 g l^{-1} dye and 0.75 g l^{-1} dye at both 1.9 and 3.8 g l^{-1} starch (Table 6.2). Therefore the results of Expt 4.5 also indicate that COD removal was affected by dye COD. The highest overall percentage COD removal (88%) was obtained in Expt 4.3 (Figure 6.3). This Expt showed the biggest contribution to COD removal by activated sludge treatment. Therefore the usefulness of the aerobic stage was proved in treatment of textile effluent when the organic load of the anaerobic effluent was high. A fixed emission standard of 125 mg l^{-1} COD is required in the UK under the Urban

Wastewater Treatment Directive for populations >2000 (Gray, 1999). Therefore the final effluent would need to undergo further treatment prior to discharge.

With the exception of Expt 4.2 the anaerobic COD removal was similar to or greater than that achieved by Zhu *et al.* (1994) and An *et al.* (1996) (Section 5.3.3.1). In all Expts the aerobic COD removal was in excess of the 2.9-8.9% achieved in Expts 3.2-3.4 (Section 5.3.3.1), and in Expt 4.3 was similar to the 23-30% found by Zhu *et al.* (1994) and An *et al.* (1996). The overall COD removal in all Expts, with the exception of Expt 4.2, was similar to or greater than that obtained by Zaoyan *et al.* (1992) at 77-88%, and the removal achieved in Expt 4.3 (88%) was similar to that reported by Zhu *et al.* (1994) and An *et al.* (1996). This showed that under most experimental conditions in Experiment 4 the UASB performed well and in a manner consistent with treatment of other textile wastes. It also showed this work to be comparable with that of other authors. The overall COD removal obtained in Expt 4.2 was poorer than published results indicating that this STE composition (Table 6.1) was less degradable than that used by other authors.

The BOD measurements can be seen in Table 6.2 while Figure 6.4 illustrates the percentage reduction of BOD by each stage. The final BOD values were relatively high (65-157 mg l^{-1}) compared to the Royal Commission Standards for discharge (20 mg BOD l^{-1}). This shows that biodegradable material was still present in the final effluent and that tertiary treatment would therefore be required before final discharge. As with the COD, most BOD was removed in the UASB. In Expt 4.3 the aerobic stage played a greater role in BOD removal than in the other Expts. This confirmed that the aerobic stage compensated for the lower removal of organic material by the UASB when the loading rates were high.

The highest final BODs were obtained at 3.8 g l^{-1} starch (Expts 4.3 and 4.4) and the final BOD in Expt 4.4 was significantly higher than that of all other Expts (95-99.9% CI). This differed from the COD results, when the highest final value was found in Expt 4.2. This indicated that either the final effluent of Expt 4.2 was less biodegradable than that of

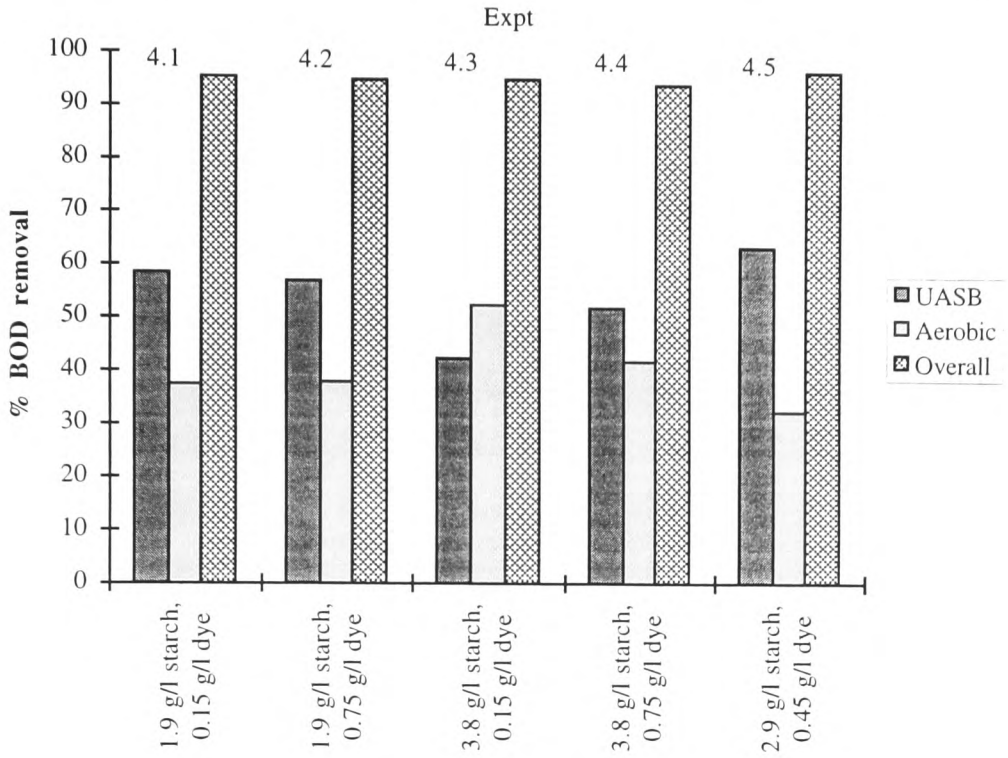


Figure 6.4 Percentage BOD Removal By UASB, Aerobic Stage And Overall For Each Expt.

Expt 4.4 or that the error of the BOD affected the results. The poor biodegradability of the dye, contributing 17% to the projected COD in Expt 4.2, indicates that the former is likely to be correct. There was no significant difference in the final BOD in Expt 4.5 compared to those obtained in Expts 4.1 and 4.2 at 1.9 g l^{-1} starch. Therefore despite the presence of 2.9 g l^{-1} starch in the STE used in Expt 4.5, the BOD was removed to the same concentration as in the Expts with only 1.9 g l^{-1} starch. A BOD removal of 94-96% was achieved in each case for Expts 4.1-4.5 (Figure 6.4), similar to that achieved by Zaoyan *et al.* (1992).

The maximum STE COD:BOD in Expts 4.4-4.5 was 1.94 (Expt 4.2; Table 6.2). This showed that the STE was readily biodegradable. The COD:BOD of the final effluent exceeded that of the STE showing that biodegradable material was removed during treatment, as also occurred in Expts 3.1-3.4 (Section 5.3.3.1). An *et al.* (1996) reported wastewater to be biodegradable at BOD:COD of above 0.25, when studying effluent from dye manufacture. The BOD:COD of the STE exceeded this value for every Expt (Table 6.6). It was therefore classified as biodegradable, thus proving the accuracy of the

anaerobic biodegradability tests (Section 3.4.2). It has also been reported that if the BOD:COD of dye wastewater increases after anaerobic treatment then the waste has been made more amenable to degradation. This was attributed to a change in the dye's molecular structure (An *et al.*, 1996). The BOD:COD ratios for STE and UASB effluent increased by 19-47% in Expts 4.1, 4.3 and 4.4 (Table 6.6). There was obviously a corresponding decrease in the COD:BOD ratios (Table 6.2). Therefore it is likely that the dye was cleaved to give rise to aerobically biodegradable substances, such as aromatic amines. The ratios decreased after aerobic treatment showing that the biodegradable material had been removed. In Experiment 3 a decrease in COD:BOD, corresponding to an increase in BOD:COD, was not observed subsequent to anaerobic treatment. The exception to this was Expt 3.1, when the UASB was not operating well (Table 5.4). Expt 3.3 had the same STE composition as Expt 4.1 and therefore similar observations would have been expected in both cases. This indicates that adaptation of UASB operation may have occurred. The small increase in the COD:BOD in Expts 3.2-3.4 following anaerobic treatment (Table 5.4) may have been attributable to biodegradability of STE being increased to a lesser extent than in Experiment 4.

Table 6.6 BOD:COD For STE, UASB Effluent And Final Effluent Together With The Percentage Change After Anaerobic Treatment.

Expt	STE	UASB effluent	Final Effluent	% anaerobic increase	% aerobic decrease*
4.0	0.47	0.43	0.03	-7.9	93.2
4.1	0.67	0.83	0.15	23.7	82.1
4.2	0.51	0.46	0.08	-10.7	83.0
4.3	0.54	0.79	0.23	46.7	70.8
4.4	0.65	0.78	0.19	18.7	75.1
4.5	0.59	0.56	0.09	-5.4	83.4

*from UASB effluent

6.3.3.2 Gas Measurements.

A maximum concentration of 1.2% H_2S was detected in the biogas. Therefore the total sulphide concentration in the liquid phase was 62 mg l^{-1}, based on a pH of 6.9, according to the relationship between H_2S gas and total sulphide in the liquid phase (Section

1.5.2.1). The mean pH in the UASB was 7.3 in Experiment 4. When the pH factor was compensated for using equations given by Speece (1996) the total sulphide concentration was calculated to be 93 mg l^{-1}. This was below the 0.1-0.8 g l^{-1} sulphide cited as causing toxicity (Section 1.5.2.1) and confirms that the UASB was unlikely to be suffering from toxicity. This concurs with the conclusions of Section 3.4.1 when sulphate concentrations were deemed not to be problematic.

The rate of biogas production ranged from 539-972 mls gas l^{-1} reactor d^{-1} (Table 6.7). This again showed that high CODs were associated with high rates of gas production, as in Section 5.3.3.2. The methane yield ranged from 0.25-0.33 l CH_4 g^{-1} COD removed. Therefore once more the theoretical yield was not achieved, probably for the reasons cited in Section 4.3.2. The mean percentage carbon dioxide in biogas at steady state was 26-27% in Expts 4.1, 4.2 and 4.5 but 29-30% during Expts 4.3 and 4.4. This higher percentage carbon dioxide was concurrent with an increase in TVFAs in the UASB (Table 6.3; Section 6.3.3.3). This indicated either that more sodium bicarbonate was degraded to counteract the increase in TVFAs, or that methanogenesis was inhibited resulting in less acetate, carbon dioxide and hydrogen being converted into methane. The result of either is the generation of higher concentrations of CO_2, indicating that the digester was not stable during Expts 4.3 and 4.4.

Table 6.7. Gas Production, Percentage Carbon Dioxide And Methane Yield For Expts 4.1-4.5.

Expt	l gas produced l^{-1} reactor d^{-1}	CO_2 (%) in biogas	Methane yield (l CH_4 g^{-1} COD removed)
4.1	539	26.9	0.27
4.2	564	25.8	0.30
4.3	792	29.6	0.25
4.4	972	29.3	0.30
4.5	854	26.13	0.33

6.3.3.3 Volatile Fatty Acids and Bicarbonate Alkalinity.

In Expts 4.1, 4.2 and 4.5 the mean TVFA concentration was below 400 mg l^{-1} indicating that the reactor was not stressed. Propionic acid concentrations were 25-80 mg l^{-1}. In Expts 4.3 and 4.4 at 3.8 g l^{-1} starch the reactor contained higher quantities of VFAs including high concentrations of propionic acid (Table 6.3), suggesting instability. The concentrations of propionic acid in Expt 4.5 were intermediate between those in Expts 4.1-4.2 and those in Expts 4.3-4.4, but there was no increase in TVFA. The high standard deviations in Expts 4.3 and 4.4 were due to the different response of the UASB to repeats of the same Expt. The first repeat of Expt 4.3 contained a higher concentration of TVFAs, and in Expt 4.4 the second repeat had the higher concentration. This made accurate comparison of the VFA results from these two Expts difficult. However, it appeared that when the dye concentration was increased to 0.75 g l^{-1} at the same starch concentration (Expt 4.1 compared with 4.2, and 4.3 compared with 4.4) there was no effect on TVFA concentration. When the starch concentration was increased to 3.8 g l^{-1} at the same dye concentration (Expt 4.1 compared with 4.3, and 4.2 compared with 4.4), TVFA concentration increased. As the TVFA concentration did not increase at 2.9 g l^{-1} starch it was concluded that 0.15-0.75 g l^{-1} of this dye did not affect methanogenesis but starch concentrations in excess of 2.9 g l^{-1} did. Therefore the maximum sludge loading rate to the anaerobic reactor was concluded to lie between 0.12 (Expt 4.5) and 0.15 (Expt 4.4) g COD g^{-1} VS d^{-1}, corresponding to a B_v of 3.14-3.9 g COD l^{-1} reactor d^{-1}. The results obtained in Experiment 1 indicated that a B_v of 3.47 g COD l^{-1} d^{-1} exceeded the tolerance of a UASB with a higher concentration of biomass l^{-1} digester (Section 4.3.3). Therefore the UASB tolerance can be narrowed down to 3.14-3.47 g COD l^{-1} d^{-1}. The 0.12 g COD g^{-1} VS d^{-1} was in excess of the B_x tolerated in Experiment 1, as was also observed in Section 5.3.3.3. Therefore it may be possible for the UASB to become adapted to higher loading rates, hence increasing the tolerance limits.

The pH of the UASB was similar in Expts 4.1-4.5 at 7.2-7.3. The bicarbonate alkalinity of the UASB increased in Expts 4.3 and 4.4 to 1972-2054 mg l^{-1} compared to 1786-1812 mg l^{-1} in Expts 4.1 and 4.2, despite higher TVFA concentrations in the UASB. This was due to the higher quantity of starch in STE used in Expts 4.3 and 4.4. Due to

the method of hydrolysis high starch concentrations gave rise to more NaOH in the STE, which provided more buffering capacity. The BA in Expt 4.5 was higher again at 2215 mg l^{-1} although there was no apparent reason for this. When the TVFA:BA was examined for each Expt the ratios were found to be 0.18, 0.13, 0.49, 0.35 and 0.17 for Expts 4.1-4.5 respectively. Hence in Expts 4.3 and 4.4 the TVFA:BA was in excess of that found in stable systems (Section 1.5.2.1). It therefore appeared that 3.8 g l^{-1} starch at a 1 d HRT was in excess of the maximum tolerance of the UASB. However, it appeared that the UASB had adapted better by Expt 4.4 compared to Expt 4.3 as the TVFA:BA was lower in Expt 4.4. It may therefore be possible to adapt the UASB to high starch loading rates.

6.3.3.4 Colour Removal.

Most colour removal occurred anaerobically as observed in Sections 4.3.4 and 5.3.3.4. The anaerobic colour removal in Expts 4.3-4.5 was within the range cited by the manufacturer (Section 3.2.1) and similar to that achieved by Boe *et al.* (1993). However, it was lower than that achieved by An *et al.* (1996) and Razo-Flores *et al.* (1997) (Section 5.3.3.4). Therefore the UASB used here can be said to remove colour to an extent comparable with other UASBs. Anaerobic colour removal in Expts 4.1 and 4.2 was below that cited by the authors listed here. In Experiment 4, unlike Experiment 3 (Section 5.3.3.4) higher anaerobic removal of true colour was not observed at higher dye concentrations. The addition of high concentrations of starch was also observed to affect colour removal in this Experiment. Most anaerobic colour removal is thought to occur by means of degradation (Section 1.5.2.1.1). The UASB was operated on STE during Expt 4.0, prior to Expts 4.1-4.5, and hence the adsorption sites should have already reacted with the dye. Therefore degradation was the most likely cause of colour removal.

The aerobic colour removal in Experiment 4 was in excess of the approximately 10% cited by the manufacturers (Section 3.2.1), Boe *et al.* (1993) and An *et al.* (1996) (Section 5.3.3.4), with the exception of Expt 4.2. A maximum of 77% overall colour removal was achieved in Expt 4.3, giving the lowest final true colour value (0.21 abs units; Table 6.4).

The overall colour removal of Expt 4.1 was close to that of Loyd *et al.* (1992) while that of Expts 4.3-4.5 was closer to that of Zaoyan *et al.* (1992), with Expt 4.3 being slightly in excess of this (Section 5.3.3.4). Colour removal by combined treatment was lower than that achieved by An *et al.* (1996), as in Experiment 3. Therefore it can be said that aerobic colour removal was, in most cases, in excess of that predicted by the manufacturers, and similar to that obtained by authors treating different textile wastes. The final effluent was still coloured and would therefore require further treatment prior to discharge.

Figure 6.5 shows the percentage overall colour removal and dye concentration in the STE plotted against the starch:dye ratio (Table 6.1). It was seen that the optimum starch:dye ratio for overall colour removal varied with the dye concentration present in the STE. Expt 4.5 exhibited similar colour removal to Expt 4.3 (56% compared to 59%) although the starch:dye ratio in Expt 4.3 was nearly four times greater (Table 6.1). This further indicated the role of dye concentration in the STE in determination of the optimum starch:dye ratio. At the same dye concentration (Expts 4.2 with 4.4, and 4.1 with 4.3) the presence of extra starch greatly increased colour removal. Therefore Expts 4.3 and 4.4 showed superior colour removal to Expts 4.1 and 4.2. From the relationship between azo bond cleavage and electron availability from an organic source (Section 1.5.2.1.1), it appears that the addition of extra starch provided an increased source of reducing equivalents hence enabling more dye to be degraded. In Experiment 3 halving the starch concentration from 1.9 to 0.95 g l^{-1} had little effect on colour removal (Expts 3.3 and 3.4; Table 5.5). However, in Experiment 4 at 0.15 g l^{-1} dye, doubling the concentration of starch from 1.9 to 3.8 g l^{-1} (Expt 4.1 and 4.3) increased colour removal. This supports the hypothesis that starch concentrations must exceed a certain concentration before colour removal is enhanced (Section 5.3.3.4).

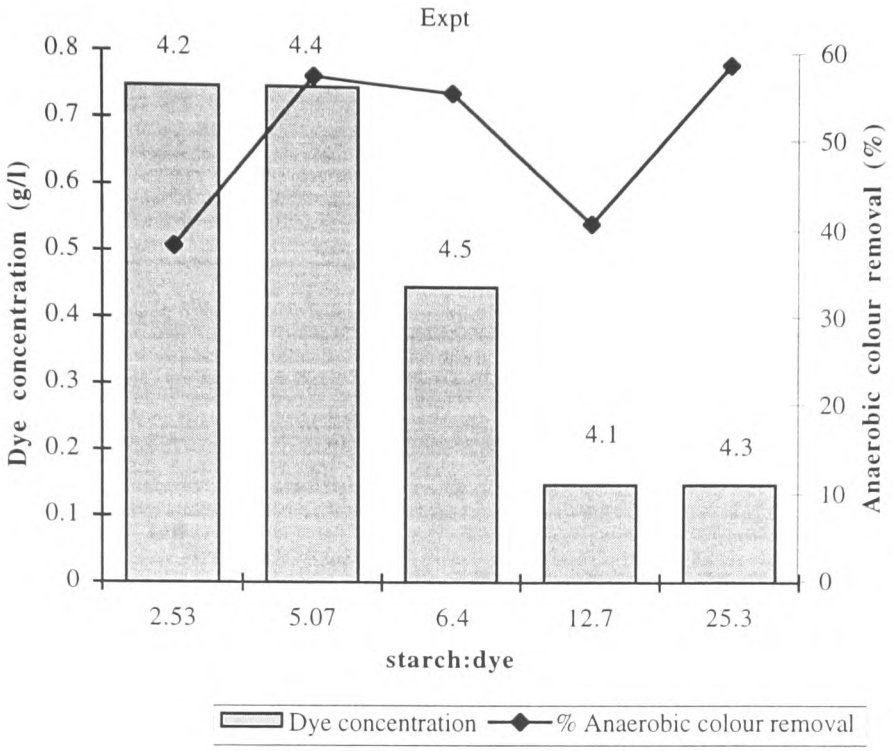


Figure 6.5 Per Cent Colour Removal And Dye Concentration In STE vs The Starch:Dye Ratio.

As the addition of starch increases colour removal, it is recommended that if anaerobic colour removal efficiency decreases, starch to a maximum sludge loading rate of 0.12 g COD g^{-1} VS d^{-1} (B_v: 3.14 g COD l^{-1} d^{-1}) to 0.15 g COD g^{-1} VS d^{-1} (B_v: 3.90 g COD l^{-1} d^{-1}) be added. At the latter loading rate the tolerance limit of the UASB is reached as evidenced by increase in TVFA concentration (Section 6.3.3.3) and therefore this loading rate should be used for short periods only.

6.3.3.5 Aerobic Stage.

The sludge replaced on day 57 was not acclimatised to the UASB reactor effluent. However, the MLSS remained high at approximately 4 g l^{-1} during the next 10 days (Expts 4.1 and 4.3). The MLSS then decreased over the next 21 days (Expts 4.1 and 4.2) to approximately 1 g l^{-1} (Figure 6.2). Over the following 5 weeks of experiments it was found that at 3.8 g l^{-1} starch (Expts 4.3 and 4.4) MLSS increased, and at 1.9 g l^{-1} starch (Expt 4.1) the MLSS fell, giving a F:M much higher than for a well performing activated sludge plant (Table 6.5). This suggested that biomass growth in the activated sludge stage

was limited by carbon source. Therefore the UASB overload led to increased production of MLSS in these Expts and to an increased role of the activated sludge system in removing COD. However, some bulking occurred at 3.8 g l^{-1} starch.

When the activated sludge stage was supplemented with a concentrate of OECD synthetic sewage (Expt 4.5) the MLSS concentration was maintained at around 2.43 g l^{-1} (Table 6.5), with a mean sludge age of 13.4 days. The concentrated OECD waste had a COD of 10.7 g l^{-1} and therefore supplied 15 g COD d^{-1} to the aerobic stage, or 29% of the total COD entering the activated sludge tank, in Expt 4.5. An F:M of 0.42 g BOD g^{-1} MLSS d^{-1} was obtained in Expt 4.5 based on the UASB effluent BOD, and not taking account of the OECD waste BOD. Tang *et al.* (1995) found a COD:BOD of 2.2:1 for domestic sewage. Using this ratio the OECD waste was calculated to supply an additional 0.14 g BOD g^{-1} MLSS d^{-1}, giving a total of 0.56 g BOD g^{-1} MLSS d^{-1}. This was a high F:M but no bulking was observed. Therefore feeding the activated sludge stage with OECD synthetic sewage enabled good operation of the activated sludge stage. This agrees with reports cited earlier regarding addition of domestic sewage to textile effluent enhancing its degradation (Section 1.5). It must be noted that to achieve true steady state in the aerobic stage as well as the anaerobic stage it would have been necessary to operate for more than 3 sludge ages. This would have more than doubled the duration of the experimental programme.

6.3.4 Amines.

6.3.4.1 Respiration Inhibition Tests.

The STE and final effluent were found to have undetectable toxicity in a 3-hour respiration inhibition test. The UASB effluent had a 3-hour respiration inhibition of 17.9%. This showed that anaerobic degradation of the STE produced metabolites that were toxic to at least some of the aerobic organisms used in the test. The dye was the only substance present in the STE that could produce toxic by-products by means of anaerobic digestion. The toxicity was eliminated after aerobic treatment indicating that

the products causing toxicity in the respiration inhibition test were degraded aerobically. This suggested that the dye was not simply adsorbed to anaerobic biomass but actually degraded anaerobically to amines. Hence at least some of the colour removal observed was attributable to dye degradation. It was also probable that some of the COD removal in the aerobic stage was attributable to degradation of the dye metabolites, as reported by Field *et al.* (1995).

6.3.4.2 Amine Analysis.

Anaerobic colour removal is principally attributed to biodegradation (Section 1.5.2.1.1). That being the case, amines should be present in anaerobic effluent from treatment of textile waste. Although the detection of simple aromatic amines is relatively easy, it becomes analytically much more complex when additional groups (e.g. $-SO_3$, -OH, -COOH, -Cl) are present in their aromatic structure. When, as in this study, azo-dye removal only needs to be demonstrated qualitatively it is helpful to use simple analytical methods. The best known method for the qualitative detection of aromatic amines is the diazonium coupling reaction (March, 1968; Norwitz and Keliher, 1986). However, because this method is a colorimetric assay giving a red colour, it is not suitable for use with red samples such as those resulting from biodegradation of PROCION Red H-E7B. Therefore a simple HPLC-based procedure was used, coupled with total organic nitrogen (TON) measurements, to determine whether colour was removed by means of adsorption or dye degradation during anaerobic treatment, and to determine the fate of anaerobic degradation products in aerobic treatment.

Several peaks were present in all chromatograms recorded by HPLC-UV analysis with the 250/3 NUCLEOSIL 100-5 C18 AB column (Section 2.5.1.2). However, none corresponded to any of the 16 aromatic amines identified by the column manufacturers as being commonly formed during dye degradation. The presence of the unidentified peaks can be explained by the hypothesis that during biological degradation of the dye several derivatives containing amino groups ($-NH_2$) were formed. This explanation agrees with the results of Carliell *et al.* (1994b, 1995) who demonstrated that during anaerobic

degradation of PROCION Red H-E7B, several amino-derivatives bearing groups such as -OH and -SO_3 were formed as azo bonds were reduced. The findings with the C18 SUPELCOSIL HPLC column are shown in Figure 6.6.

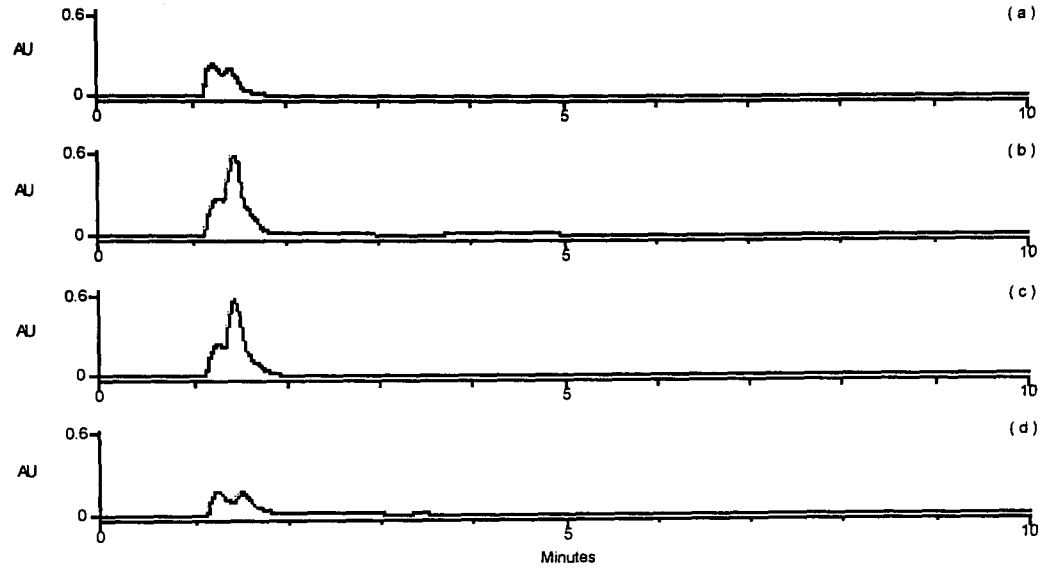


Figure 6.6 HPLC-UV Chromatograms Obtained Using A C18 SUPELCOSIL Column. [(a) STE; (b) UASB Effluent; (c)Aerobic Feed (i.e. 30 L Of UASB Effluent: 1.4 L Of OECD Synthetic Sewage); (d) Final Effluent]. AU - absorbance units.

Figure 6.6 shows the HPLC chromatograms of STE, UASB effluent, Aerobic Feed (30 l of UASB effluent + 1.4 l of OECD waste) and final effluent. Figures 6.6(a) and 6.6(b) proved that UV absorbing by-products were formed during the anaerobic treatment of the UASB feed (total UV detectable area increased from (a) to (b)). Comparison of chromatograms Figure 6.6(b) and 6.6(c) proved that the synthetic sewage addition to the UASB effluent did not modify the HPLC chromatogram as chromatograms (b) and (c) were the same. Furthermore, comparison of chromatograms Figure 6.6(c) and 6.6(d) qualitatively proved that partial degradation of the UV-absorbing by-products formed during anaerobic treatment occurred in the aerobic stage (the total UV detectable area decreased from Figure 6.6(c) to 6.6(d)). The total organic nitrogen (TON) decreased from 144 mg N l^{-1} prior to activated sludge treatment to 108 mg N l^{-1} subsequent to such treatment. Taking the HPLC-UV results into account together with the decrease in TON it can be reasonably concluded that during the aerobic stage some degradation of nitrogen-

containing aromatic derivatives, likely to be aromatic compounds bearing amino-groups, took place with associated mineralisation of organic nitrogen.

The chromatograms in Figure 6.7 were recorded at a higher absorbance sensitivity (Y axis), and UV absorbing substances eluting in the range 2-5 minutes were examined, providing additional information about the degradation by-products formed. The chromatogram for sample (b) is not shown in Figure 6.7 because, as in Figure 6.6, it was the same as (c).

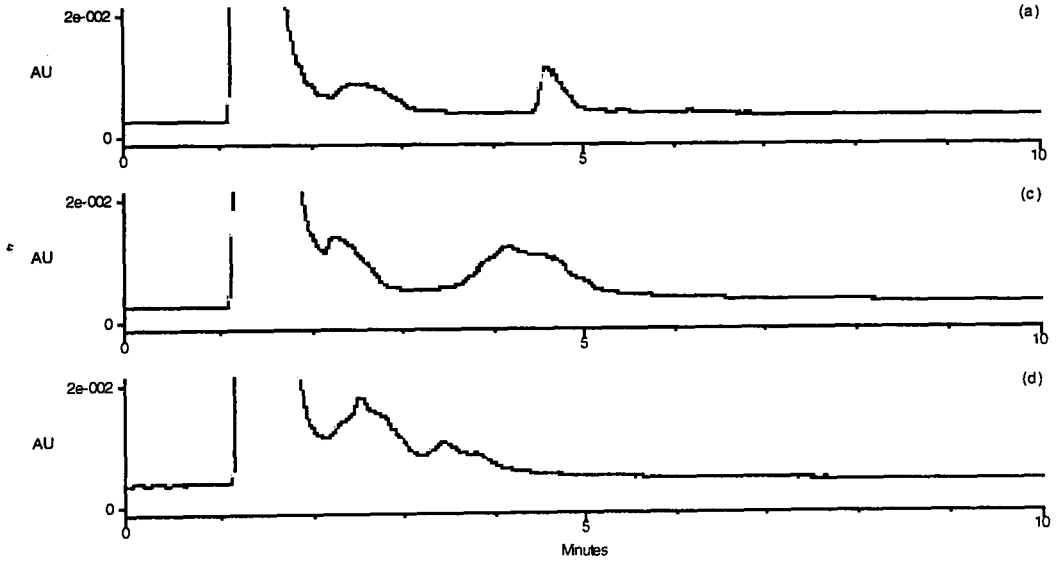


Figure 6.7 HPLC-UV Analyses Obtained Using A C18 SUPELCOSIL Column And A High UV-Absorbance Sensitivity. [(a) STE; (c)Aerobic Feed (i.e. 30 L Of UASB Effluent: 1.4 L Of OECD Synthetic Sewage); (d)Final Effluent]. AU - absorbance units.

In Figure 6.7, as in Figure 6.6, comparison of chromatograms (a) and (c) showed that after anaerobic treatment the UV detectable area increased. This confirmed the formation of additional aromatic by-products. Furthermore, the shift of the UV detectable area in (c) towards lower retention times meant that the by-products were more polar than the parent compounds shown in (a). This experimental evidence, once again, agreed very well

with previous results that more rigorously demonstrated that the anaerobic degradation of reactive azo dye produces several aromatic and ionic (i.e. highly polar) by-products (Carliell *et al.*, 1994b, 1995).

Given the reported toxicity and respiration inhibition effects associated with amino-derivatives (Section 1.5.2.1.1), the respiration inhibition of aerobic bacteria caused by UASB effluent (Section 6.3.4.1) supports this observation. Figure 6.7 (c and d) also showed that when such by-products were degraded during subsequent aerobic treatment, they formed less aromatic (the UV detectable area decreases in Figure 6.7d) and more polar (the UV detectable area is shifted towards lower retention times in Figure 6.7d) end products. If the hypothesis that amino-derivatives are formed during the anaerobic stage is accepted, it is reasonable to assume that their degradation in the aerobic stage would result in the formation of oxidised and very polar derivatives (e.g. aldehydes, carboxylic acids) having a lower aromaticity as suggested by Nörtemann *et al.* (1986) when studying 6-aminonaphthalene-2-sulphonic acid degradation. By-products exhibiting such characteristics were observed in Figure 6.7. Together with the decrease in TON through aerobic treatment, it can be reasonably concluded that during the aerobic stage some degradation of nitrogen-containing aromatic derivatives (likely to be aromatic compounds bearing amino-groups) took place with mineralisation of organic nitrogen. The dye therefore acted as a source of carbon and nitrogen for the activated sludge stage.

It was concluded that the results from simple HPLC-UV analyses coupled with TON measurements, colour removal, COD removal and respiration inhibition tests qualitatively demonstrated that aromatic amino-derivatives were formed during anaerobic treatment and were degraded into more polar, non-aromatic by-products during the following aerobic stage. It was therefore concluded that the dye was degraded to amines by means of anaerobic digestion, which were then removed by means of activated sludge treatment. Anaerobic biodegradation of dyes is cited in the literature as generating aromatic amines when colour removal occurs by cleavage of the azo bond. The primary reason for using an aerobic stage was to remove these amines. Hence anaerobic treatment enhanced aerobic biodegradation and the activated sludge stage fulfilled the function for which it was intended.

6.4 Conclusions.

The treatment efficiency of the system at 1.9 g l^{-1} starch and 0.15 g l^{-1} dye remained constant despite 7 intervening 1-week periods of operation at other starch:dye ratios. This indicated that the system could tolerate step changes without any change in effectiveness of operation.

The UASB improved the treatability of the effluent, the BOD:COD rising by up to 47%. A BOD removal of 94-96% was achieved in Expts 4.1-4.5 and the maximum COD removal was 88% with a lowest final COD of ~440 mg l^{-1}. The aerobic stage compensated for the lower percentage COD removal by the UASB when the loading rates were high. However, addition of synthetic sewage to the aerobic reactor was required to maintain MLSS. This gave F:M ratios in excess of those recommended for activated sludge.

A maximum 77% overall colour removal was achieved at STE starch and dye concentrations of 3.8 and 0.15 g l^{-1}. Most colour removal occurred anaerobically. The optimum starch:dye ratio for overall colour removal varied with the initial dye concentration used. It appeared that the starch had to exceed a certain minimum concentration before any noticeable improvement in colour removal was observed.

At 0.15 and 0.75 g l^{-1} dye a starch concentration of 3.8 g l^{-1} gave an improvement in colour removal compared to a starch concentration of 1.9 g l^{-1}. However, the UASB reactor began to show signs of instability. The UASB could, however, tolerate STE starch concentrations of 2.9 g l^{-1} at a 1 d HRT. The maximum tolerable starch concentration therefore was between 2.9 and 3.8 g l^{-1} at this UASB HRT. It is recommended that if colour removal efficiency decreases, carbohydrate to a maximum sludge loading rate of between 0.12 and 0.15 g COD kg^{-1} VS d^{-1} be added. The UASB tolerance appeared to lie between B_v 3.14-3.90 g COD l^{-1} d^{-1}. From results obtained previously it appeared that the limits could be as close as 3.14-3.47 g COD l^{-1} d^{-1} in treatment of this STE.

Anaerobic degradation of the dye used in this STE produced aromatic amines that were removed aerobically. The increase in BOD:COD after some Expts indicated that the waste was made more biodegradable by anaerobic treatment. Some aerobic COD removal was due to this aerobic degradation of the amines. A simple HPLC was suggested which, together with TON analysis, respiration inhibition tests and colour removal could be used to determine whether amines were produced and removed by means of combined anaerobic-aerobic treatment of textile effluent.

CHAPTER SEVEN - CONCLUSIONS AND FUTURE WORK.

7.1 Aims and achievements.

The aims of this research were described earlier (Section 1.6) and are restated below. The outcomes of the research in relation to achievement of these aims are also discussed.

- To generate a simulated effluent that was representative of real cotton processing effluent, while maintaining a simple composition.

A simulated cotton processing effluent was generated which was similar to real effluents in terms of COD, BOD, COD:BOD, pH, and TSS (Section 3.4.4). The simulated effluent was less complex than real textile wastewaters and contained only one dye. Therefore the principal difference between the STE and real effluents was in the spectrum. The relationship between ADMI and concentration of this dye was similar to that found in real effluents (Section 3.4.4). The simulated waste was manipulated by altering the dye and starch concentrations from 0.075-1.5 g l^{-1} and 0.95-3.8 g l^{-1} respectively.

- To investigate the possibility of using coagulation/flocculation as a pre-treatment process.

A range of coagulants/flocculants was tested (Table 2.3). However it was concluded that none of the products were suitable for treatment of this STE prior to biological treatment due to problems of toxicity, pH adjustment, high sludge volumes produced by some coagulants and long settling times associated with others (Section 3.4.5).

- To assess ITD and UASB reactors to determine which was the most suitable for anaerobic treatment of this waste type.

The UASB was concluded to be more effective than the ITD in treatment of STE as COD removal at a 2 d HRT was comparable to that of the ITD at a 2.8 d HRT (Table 4.2), while the colour removal was greater (Table 4.4). Higher B_vs were obtained in the UASB due to the higher quantity of biomass. This meant that larger volumes of effluent could be treated compared to the ITD. The principal disadvantage of the UASB was the loss of biomass over time. The problem of sludge loss might be rectified by developing a superior 3-phase separator. The ITD was superior to the UASB in retaining sludge (Section 4.3).

- To evaluate the application of a combined anaerobic-aerobic treatment system to the treatment of simulated effluent.

The volumetric loading rates achieved for the anaerobic stage were comparable with those obtained by other authors. Anaerobic sludge loading rates were below the values achieved by other authors. Biomass was lost from the UASB throughout the series of Experiments (Sections 5.3.1 and 6.3.1). Some pH adjustment of the activated sludge stage was required (Sections 4.3.5 and 5.2.1). It was necessary to feed the activated sludge stage with a concentrate of OECD synthetic sewage in order to maintain the MLSS (Section 6.3.3.5). Therefore treatment of textile effluent in conjunction with domestic sewage could give good operation of the aerobic stage. The final effluent from combined anaerobic-aerobic treatment had a BOD in excess of the 20 mg l^{-1} required under the Royal Commission Standards for discharge. The final COD was in excess of the 125 mg l^{-1} required under the Urban Wastewater Treatment Directive for populations >2000 (Section 6.3.3.1). Up to 77% of the true colour of the STE was removed by combined treatment. However some colour still remained. Therefore tertiary treatment of the effluent produced by this combined anaerobic-aerobic treatment would be required to remove excess COD, BOD and colour.

- To evaluate the effect of anaerobic and aerobic treatment on colour and COD removal.

The majority of COD was removed anaerobically (Sections 4.3.1, 5.3.3.1 and 6.3.3.1). Therefore anaerobic treatment reduced the COD load to the aerobic stage and thus reduced the quantity of aerobic sludge produced. Aerobic treatment alone (Expt 3.5) was found to remove a percentage of COD comparable to that achieved by combined anaerobic-aerobic treatment (Section 5.3.4). When step increases in load were carried out (Expts 4.3 and 4.4) or when the UASB was not operating at its optimum (Expt 3.1) the aerobic stage removed a greater quantity of COD, and thus compensated for the higher anaerobic effluent COD (Sections 5.3.3.1 and 6.3.3.1). The majority of colour was removed anaerobically (Sections 5.3.3.4 and 6.3.3.4). Aerobic treatment alone of STE was found not to remove colour (Section 5.3.4). Therefore the anaerobic stage reduced the organic loading to the aerobic stage and removed colour while the aerobic stage removed excess COD.

- To establish operating parameters for the anaerobic and aerobic stages treating different simulated textile effluent compositions.

A 1 day HRT in the UASB was suitable for treatment of STE containing 0.075-0.75 g l^{-1} dye. Longer HRTs were tested at 1.5 g l^{-1} dye in order to obtain good colour removal (Tables 4.4 and 5.3). Dye concentrations up to 0.75 g l^{-1} did not appear to affect the stability of the UASB but starch concentrations in excess of 2.9 g l^{-1} caused an increase in TVFA concentration (Section 6.3.3.3). The maximum B_v to the UASB appeared to lie between 3.14 and 3.47 g COD l^{-1} d^{-1}. However, it was possible that the UASB might become acclimatised to higher volumetric loading rates (Section 6.3.3.3). When the UASB removes most of the COD present in the feed it is recommended that the activated sludge stage be fed with OECD waste to provide 30% of the COD entering that stage (Section 6.3.3.5). The treatment efficiency of the combined anaerobic-aerobic system remained constant despite intervening periods of operation at other starch:dye ratios (Experiment 4). This indicated that the system could tolerate step changes without any alteration in effectiveness of operation (Section 6.3.2.5).

- To investigate the relationship between dye and co-substrate (starch).

Starch:dye ratios of 1.27-25.3 were tested here (Section 4.1, Tables 5.3, 5.4 and 6.1). The optimum starch:dye ratio for anaerobic colour removal varied with the dye concentration present in the STE. At 0.15 and 0.75 g l^{-1} dye a starch concentration of 3.8 g l^{-1} gave an improvement in colour removal compared to a starch concentration of 1.9 g l^{-1}. Therefore the addition of extra starch appeared to provide an increased source of reducing equivalents hence enabling more dye to be degraded. It appeared that starch concentrations must exceed a certain concentration before colour removal was enhanced (Section 6.3.3.4). It was therefore recommended that if colour removal efficiency decreases, starch to a maximum sludge loading rate to the anaerobic reactor of between 0.12 g COD g^{-1} VS d^{-1} (B_v: 3.14 g COD l^{-1} d^{-1}) and 0.15 g COD g^{-1} VS d^{-1} (B_v: 3.90 g COD l^{-1} d^{-1}) be added (Section 6.3.3.4).

- To determine whether aromatic amines were produced by anaerobic treatment and hence clarify whether anaerobic colour removal was achieved by adsorption or dye degradation.
- To discover whether such amines were removed by means of aerobic treatment.

Infrared spectrometry indicated that amines might be present in ITD effluent. However, due to the range of molecules that can give rise to vibrations at the relevant areas of the spectrum, this was not a very useful method for amine detection (Section 4.3.6). Respiration inhibition tests found that anaerobic treatment of STE produced toxic by-products which were removed aerobically (Section 6.3.4.1). HPLC-UV found that anaerobic treatment produced aromatic by-products which were more polar than the parent compounds. These were partially degraded in the aerobic stage to produce less aromatic and more polar products. Total Organic Nitrogen analysis showed that nitrogen was removed during aerobic treatment (Section 6.3.4.2). Therefore when the results of HPLC-UV, Total Organic Nitrogen analysis and respiration inhibition tests were considered it could be reasonably concluded that anaerobic degradation of PROCION Red H-E7B produced aromatic amines which were removed aerobically. Hence at least some

colour removal occurred by means of biodegradation. The combination of methods did not enable qualitative determination of which amines were present. As the amines were degraded aerobically, some aerobic COD removal was due to their degradation (Section 6.3.4.1).

7.2 Future Work.

- The combined anaerobic-aerobic treatment system used here should be applied to other dyes to determine whether the results obtained were similar.

Such work would be useful because different dyes may respond differently to anaerobic treatment, even within the same dye class. This was seen in Table 1.6 where colour removal varied with each dye, some exhibiting good colour removal and others very poor removal. Therefore the effect of combined anaerobic-aerobic treatment on other dyes could be assessed and recommendations made to overcome problems associated with decolourisation of recalcitrant dyes. This would provide additional information on the conbined treatment system prior to applying it to real wastes and indicate when granulated activated carbon (GAC) should be used prior to anaerobic treatment in order to prevent inhibition of the anaerobic stage by recalcitrant dyes or other substances present within real effluents as recommended in the EU project.

- The combined anaerobic-aerobic treatment system should be applied to real textile effluent.

The combined treatment system appeared to cope with changes in STE composition in this project as the treatment efficiency remained constant following step changes (Section 7.1). However the variation in real effluent is continuous (Section 1.4). Use of this anaerobic-aerobic treatment system with real textile effluents would permit assessment of the impact of such continuous variation on the effectiveness of treatment.

- Application of tertiary treatment of effluent of combined anaerobic-aerobic treatment.

Tertiary techniques investigated by other partners in this project included ozonation and membrane filtration. The effluent of combined anaerobic-aerobic treatment in this project did not meet COD and BOD emission standards (Section 6.3.3.1). There was also some colour remaining in the effluent (Sections 5.3.3.4 and 6.3.3.4). Should the combined anaerobic-aerobic treatment system be found suitable for treatment of real textile effluents, the tertiary techniques investigated in the EU project should be applied to the final effluent to enable the effluent to meet discharge consents.

- A better 3-phase separator design for the UASB should be investigated in order to see whether this would facilitate better retention of solids within the UASB.

Solids were lost from the UASB throughout the operating period (Sections 4.3, 5.3.1 and 6.3.1). Development of a more efficient 3-phase separator might prevent such loss of biomass. This would enable higher volumetric loading rates to obtained and hence larger volumes of effluent to be treated.

- This work should be repeated with other anaerobic reactors for comparison with the UASB.

It was found that the UASB gave better colour removal than the ITD while providing similar COD removal, although biomass was lost. Investigation of other anaerobic systems might identify a digester which could provide good COD and colour removal while maintaining its biomass concentration.

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Review

Anaerobic Treatment of Textile Effluents: a Review

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Abstract: The treatment of textile waste water is commonly carried out using biological (mainly aerobic) and physico-chemical systems. However, anaerobic bioreactors can be used to at least partially treat these effluents and provide a number of significant advantages. The most attractive feature for the treatment of textile effluents is the decolourisation of many dyes under the reducing conditions present in an anaerobic reactor. Laboratory-scale results on this particular topic are here reviewed. A second major advantage of anaerobic processing is its ability to treat wastestreams with high organic loads such as the effluents from the desizing and scouring operations currently employed in the textile manufacturing industry. Reports on successful, full-scale and pilot-scale plants are also reviewed and some limitations are discussed. © 1998 Society of Chemical Industry

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Key words: textile effluent; colour removal; dye degradation; anaerobic treatment

NOTATION

ABS	Alkylbenzenesulphonate
AE	Alkyl ethoxylate
AOX	Adsorbable organic halogen
APEO	Alkyl phenol ethoxylate
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CSTR	Completely stirred tank reactor
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acids
WWTP	Waste water treatment plant

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1 INTRODUCTION

The textile processing industries produce high quantities of effluent with varying composition depending on the wet processes employed.[1,2] Water consumption has been summarised by Correia *et al.*[3] and varies between 3 and 9 $dm^3\ kg^{-1}$ textile for cotton desizing and up to 334–835 $dm^3\ kg^{-1}$ textile for wool washing. Water-saving measures and treatment by membrane filtration processes for in-process water reuse tend to result in reduced flows of more concentrated effluents.[2] Recycling of the process water can, in particular, give rise to

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increasing salt levels.[4] The major pollutant types identified in textile waste water are summarised in Table 1 along with their main origin in the textile manufacturing process. Their general impact on the anaerobic (pre-) treatment option (Table 1) is discussed in more detail in Section 2. Textile effluents can have a high organic load mainly due to the removal of grease, dirt and/or sizing agents in the desizing and scouring steps. An anaerobic pre-treatment step could be a cheap alternative compared with aerobic systems as expensive aeration is omitted and problems with bulking sludge are avoided. Probably the most cumbersome partial waste flows of textile processing come from the dyehouse as water-soluble, residual dyes are difficult to remove in conventional treatment plants. The possibility of reductive decolourisation of these flows is a main feature supporting the anaerobic option, the subject of extensive research (see Section 2.2). Another problem originating mainly in the dyehouse is the nutrient load due to the need for dyebath additives. Also the use of large amounts of alkali in the bleaching, desizing, scouring and mercerising steps, as well as in reactive dyeing, can have a strong impact on reactor performance and costly pH adjustments may be necessary. These four basic topics (organic load, colour, nutrients, pH and salt effects) are each considered in detail in Section 2. Sulphur, toxicants and refractory organics are considered jointly as their main impact on an anaerobic stage is their inhibitory effect. Anaerobic treatment of textile effluents is not yet well established, although some successful pilot-scale and full-scale plants have been reported and are discussed in Section 3.

2 ANAEROBIC TREATMENT IN RELATION TO TEXTILE EFFLUENT COMPONENTS

2.1 Organic load

Scouring and desizing effluents are major contributers to the organic load in textile effluents. Traditional sizes such as starches and their derivatives are readily biodegradable under aerobic and anaerobic conditions. However, bulking of activated sludge occurs frequently if a large proportion of the wastestream consists of desizing waste water.[6] The use of recyclable sizes such as polyvinyl alcohol (PVA) is a viable option for integrated

TABLE 1
Major Pollutant Types in Textile Wastewaters, Their Origin and Relevance/Impact in Biological Treatment[3,5]

Pollutants	*Major chemical types*	*Main processes of origin*	*Major relevance/impact on biological treatment*
Organic load	Starches, enzymes, fats, greases, waxes, surfactants	Desizing Scouring Washing	High demand on aeration systems Activated sludge bulking problems
	Acetic acid	Dyeing	
Colour	Dyes, scoured wool impurities	Dyeing Scouring	Insufficient removal in bioreactors
Nutrients (N,P)	Ammonium salts, urea, phosphate-based buffers and sequestrants	Dyeing	Not removed in anaerobic processes Increased complexity and sensitivity of aerobic processes (biological nutrient removal required)
pH and salt effects	NaOH, mineral/organic acids, sodium chloride, silicate, sulphate, carbonate	Scouring Desizing Bleaching Mercerising Dyeing Neutralisation	Inhibition/collapse of bioreactors
Sulphur	Sulphate, sulphide and hydrosulphite salts, sulphuric acid	Dyeing	Sulphate-reduction in anaerobic reactors
Toxicants	Heavy metals, reducing agents (e.g. sulphide), oxidising agents (e.g. chlorite, peroxide, dichromate, persulphate), biocides, quaternary ammonium salts	Desizing Bleaching Dyeing Finishing	Inhibition of sensitive microbial groups (nitrifiers, methanogens) in bioreactors
Refractory organics	Surfactants, dyes, resins, synthetic sizes (e.g. PVA), chlorinated organic compounds, carrier organic solvents	Scouring Desizing Bleaching Dyeing Washing Finishing	Insufficient removal in bioreactors Possible accumulation in biomass aggregates/films, leading to inhibition

companies and can give organic load reductions of up to 90% in the wastewater from desizing operations.[2,7] Anaerobic treatment produces a much smaller volume of sludge when compared with aerobic treatment and no aeration is needed, a factor which represents a major cost in aerobic treatment, given the high BOD levels involved. This gives the anaerobic digestion process a potential economic advantage.

An upflow anaerobic filter gave good performance at laboratory scale treating the wastewater from a desizing and scouring department of a cotton blend factory.[8] The warm wastewater (35–40°C) had a COD load of 3000–7000 mg dm^{-3} due to natural impurities, processing impurities and the sizing materials used. No dyes or other possible toxic substances were mentioned by the author. The maximum COD loading was 2·75 kg COD m^{-3} day^{-1} with COD removal ranging from 60 to 90%. It was suggested that appreciable savings could be made in comparison to a traditional chemical treatment with subsequent aerobic system and sludge disposal. Trials have also been conducted[9,10] with a wastewater from cotton dyeing and finishing processes with COD values of 600–1200 mg dm^{-3}. The maximum loading rate with an anaerobic expanded bed reactor was 0·63 kg COD m^{-3} day^{-1} with COD removal varying from 50 to 87%. If the loading rate was increased, biogas production stopped and COD removal decreased to 35%. The maximum loading rate with an upflow anaerobic filter was 1 kg COD m^{-3} day^{-1} with COD removal in the 50–90% range. When the load was increased to 1·3 kg COD m^{-3} day^{-1} the reactor collapsed. Due to the low loading capacities, the tested reactors were considered not to be a valuable alternative for the wastewater in question, though the authors mentioned that research would be continued with a UASB in order to be able to increase the COD loading.

Raw wool scouring is reputed to be the most polluting operation within the textile industry. Of the weight of raw wool, 30–70% is impurity consisting of suint (the natural grease in sheep's wool), soil particles and excrement which must be removed by scouring. Scouring wastes also contain large quantities of dissolved mineral salts and lipidic compounds such as steroids, long-chain fatty acids, glycerol and glycerides.[11] Substances introduced in the process include soap, detergents and alkali.[3] Treatment problems have led to the closure of some wool scouring companies in Germany and Britain.[11] Activated sludge systems are not suitable for the treatment of these effluents as their organic load is too high (3000–150 000 mg COD dm^{-3}).[12] Anaerobic sludge digestion has some application in the treatment of wool scouring wastes as it can be used to treat settled sludge from primary settling tanks and waste sludge from the activated sludge process. Wool scouring wastes are treated in Australia in open anaerobic and facultative ponds. However there are problems associated with this treatment as odours are produced in the ponds which also tend to be unsightly.[13]

An anaerobic fixed-bed upflow digester proved to be a suitable option for a wool scouring effluent in laboratory-scale tests as the first stage of a combined anaerobic–aerobic treatment process, as it removed half of the COD with a good biogas yield (0·16–0·34 m^3 kg^{-1} COD removed).[11] In other tests COD removal up to 86% was achieved with a laboratory digester with an influent of 30 500 mg COD dm^{-3} at a hydraulic retention time of 2·4 days.[12]

The removal of 70–90% grease and 60–86% COD from a wool scouring effluent by anaerobic bioflocculation with gravity settling has also been demonstrated.[14] The development of anaerobic bacteria destabilised the grease–water emulsion, thus allowing an easier phase separation. Methane production and VFA consumption were negligible under these conditions, indicating that the main mechanism of grease removal was bioflocculation, rather than anaerobic degradation. However, a high hydraulic retention time of 4–10 days was needed. The addition of a cationic flocculant after bioflocculation allowed the reduction of the hydraulic retention time to 1–2 days with grease reduction levels above 90% at laboratory scale and up to 80% at pilot scale.[15]

2.2 Colour

2.2.1 Overview

Dyes are generally very resistant to degradation under aerobic conditions.[16] Dyestuff removal currently occurs in the primary settling tank of a WWTP for the water-insoluble dye classes (disperse, vat, sulphur, azoic dyes), while the main removal mechanism for the water-soluble basic and direct dyes in conventional aerobic systems is adsorption to the biological sludge. Reactive dyes, however, adsorb very poorly to sludge and are thus major troublemakers in relation to residual colour in discharged effluents.[16,17]

Primary degradation and decolourisation of dyes with azo-based chromophores can be achieved by the reduction of the azo bond (—N=N—). This can be done by using strong reducing agents such as sodium hydrosulphite, thiourea dioxide, sodium formaldehyde sulphoxylate and sodium borohydride.[18]

Reduction of the azo bond can also be achieved under the reducing conditions prevailing in anaerobic bioreactors.[19] The same authors also reported decolourisation of dyes other than azo dyes, although to a lesser extent, and with degradation pathways not yet documented. The amines produced by the reduction of the azo dyes are colourless but they are very resistant to further degradation under anaerobic conditions. Under aerobic conditions the mineralisation of these amines can be accomplished.[19,20] Complete treatment can thus

TABLE 2
Summary of Dye Decolourisation under Anaerobic Conditions as Mentioned by various Authors

Reference	*Dye (chromophore)*	*% colour removal*	*Initial concentration*	*Retention time*	*Culture conditions and remarks*
19	Mordant Blue 13 (mono azo)	83	100 mg dm^{-3}	42 days	Interlaboratory exercise; the presented figures are means of the reported values; batch studies in sealed bottles (0·5 dm^3) with an artificial test medium; inoculated with digester sludge at 35°C; mono and diazo dyestuff are readily biodegradable; polyazo, anthraquinone and the tested miscellaneous dyestuffs are less likely to be degraded
	Mordant Black (mono azo)	77			
	Basic Red 18 (mono azo)	92			
	Acid Yellow 151 (mono azo)	88			
	Direct Red 7 (diazo)	92			
	Acid Red 114 (diazo)	62			
	Direct Blue 15 (diazo)	83			
	Direct Yellow 12 (diazo)	75			
	Reactive Black 5 (diazo)	81			
	Acid Blue 113 (diazo)	94			
	Direct Black 19 (polyazo)	51			
	Direct Black 22 (polyazo)	61			
	Reactive Blue 19 (anthraquinone)	70			
	Acid Blue 80 (anthraquinone)	7			
	Acid Blue 25 (anthraquinone)	67			
	Basic Blue 22 (anthraquinone)	62			
	Direct Yellow 11 (stilbene)	53			
	Reactive Blue 21 (phthalocyanine)	36			Reactive Blue 21 is a Cu-containing dye
	Basic Blue 3 (oxazine)	62			
	Acid Orange 3 (nitro)	62			
	Basic Yellow 28 (methine)	35			
28	MY3 (CI 14095)(azo)	51	0·5 mmol dm^{-3}	72 h	Inoculated with a mixed bacterial community; 17 cm^3 test tubes; at 30°C; in the presence of 1800 mg dm^{-3} glucose all azo dyes were completely metabolised, except Acid Yellow 23
	Acid Red 27 (azo)	37			
	4-Hydroxyazobenzene-4′-sulphonic acid (azo)	43			
	Acid Yellow 23 (azo)	6			
	Acid Yellow 21 (azo)	98			
29	Acid Red 42 (azo)	62	80 mg dm^{-3}	16 h	UASB (15 dm^{-3}); glucose-based medium with COD 2500 mg dm^{-3}; COD removal 70%; Disperse Blue 56 made the reactor collapse
	Direct Red 80 (azo)	81	40 mg dm^{-3}		
	Disperse Blue 56 (anthraquinone)	0	30 mg dm^{-3}		
30	Reactive Yellow 16 (azo)	80–90	100 mg dm^{-3}	6·5 h	Batch studies in sealed serum bottles (0·120 dm^{-3}); assay medium consisted of 1 g dm^{-3} glucose in a phosphate buffer at 52°C; inoculum was digester sludge from wastewater works receiving textile effluents; [a] no exact concentrations given, the commercial printing dye solution was diluted 1000-fold; authors attributed the absence of colour removal with Reactive Yellow 95 to inhibitory compounds in the printing solution
	Reactive Red 198a (azo)	85–90	100 mg dm^{-3}	2 h	
	Reactive Red 141 (azo)	85–90	100 mg dm^{-3}	4·5 h	
	Reactive Blue 220 (azo)	90–95	100 mg dm^{-3}	1 h	
	Reactive Yellow 95 (azo)	0	1 : 1000[a]	—	
	Reactive Orange 12 (azo)	90–95	1 : 1000	23 h	
	Reactive Red 218 (azo)	90–95	1 : 1000	32 h	
	Reactive Orange 13 (azo)	85–90	1 : 1000	50 h	
	Reactive Red 24 (azo)	90–97	1 : 1000	32 h	
	Reactive Brown 11 (azo)	90	1 : 1000	23 h	
	Reactive Black 39 (azo)	70–75	1 : 1000	5·5 h	
	Reactive Black 5 (diazo)	80–85	100 mg dm^{-3}	4·5 h	
	Blue PB (metal complex)	98	1 : 1000[a]	2 h	
	Black SG (metal complex)	75–80	1 : 1000	7·5 h	
	Reactive Blue 49 (anthraquinone)	7–10	1 : 1000	2 h	

	Reactive Blue 38 (phthalocyanine)	40	100 mg dm^{-3}	4·5 h	
	Reactive Blue 21 (phthalocyanine)	85–90	100 mg dm^{-3}	4·5 h	
	Reactive Blue 72 (phthalocyanine)	25–30	1 : 1000[a]	50 h	
31	Acid Orange 7 (azo)	90	5 mg dm^{-3}	24 h	Labbratory-scale (2·3 dm^3) fluidised bed reactor at 25°C; synthetic wastewater with 160–185 mg COD dm^{-3}; retention times below 10 h greatly reduced the colour removal; COD removal 40%; at dye concentrations above 15 mg dm^{-3} COD removal was reduced
	Acid Orange 8 (azo)	98		12 h	
	Acid Orange 10 (azo)	81		12 h	
	Acid Red 14 (azo)	86		24 h	
32	Reactive Red 121 (diazo)	Various	Various	Various	Inhibition of anaerobic bacteria occurred at dye concentrations above 100 mg dm^{-3}; biomass previously exposed to the dye was more resistant to toxicity; glucose as carbon source enhanced biodegradation; the presence of nitrate inhibited decolourisation; sulphate did not have a detrimental effect; low redox potential gave rapid decolourisation
33	Chlorazol Yellow (diazo)	70–85	1 : 10 dilution	72 h	0·200 dm^3 anaerobic reactors, inoculated with anaerobic sludge at 30°C; 10% (v/v) chemical industry effluent from the production of optical brighteners; decolourisation favoured by highly proteinaceous media
34	Acid Yellow 17 (azo)	20	40 mg dm^{-3}	8–20 h	Laboratory-scale UASB (4·5 dm^3) medium was a glucose solution at 1000 mg COD dm^{-3}; COD removal 50–90%
	Basic Blue 3 (phenoxazine)	72			
	Basic Red 2 (acridine)	78			
35, 36	Remazol Golden Yellow RNL (azo)	78	500 mg dm^{-3}	24 h	Batch studies in sealed glass vessels, at 26°C; isolated microbial consortium *Alcaligenes faecalis* and *Commamonas acidovorans;* decolourisation dependent upon the presence of an additional carbon and energy source (yeast extract); [b] n.i. chromophore not identified
	Remazol Navy Blue GG (diazo)	80			
	Remazol Red RB (diazo)	89			
	Remazol Blue B (diazo)	76			
	Remazol Black B (diazo)	67			
	Cibracon Orange CG (n.i.)[b]	79			
	Cibracon Red C-2G (n.i.)	88			
	Disperse Navy D2GR (n.i.)	68			
	Remazol Turquoise Blue G133 (phthalocyanine)	8			Turquoise Blue is a Cu-containing dye
37	Remazol Black B (diazo)	>95	500 mg dm^{-3}	48 h	Laboratory-scale (0·125 dm^3) upflow anaerobic filter with immobilised microbial consortium (12–20°C)
38	Mordant Orange 1 (azo)	95	100 mg dm^{-3}	8 h	Laboratory-scale (0·160 dm^3) UASB at 30°C; medium was a glucose solution at 1420 mg COD dm^{-3}; 86% COD removal; glucose was a better cosubstrate for decolourisation than VFA; one of the azo-cleavage products (5-aminosalicylic acid) was completely mineralised to methane
39	Mordant Orange 1 (MO1)(azo)	>99	100 mg dm^{-3}	8 h	Laboratory-scale (0·160 dm^3) UASB at 30°C; medium with MO1 was a glucose solution at 1420 mg COD dm^{-3}; 86% COD removal; after 217 days of operation with MO1 switched to ADS, with a glucose medium at 3000 mg COD dm^{-3}; 95% COD removal; ADS was completely mineralised; after an additional 206 days of operation cosubstrate feeding was stopped; complete mineralisation of ADS was observed in the absence of any other carbon source
	Azodisalicylate (ADS)(azo)	98·8	75 mg dm^{-3}	8 h	
	Azodisalicylate (azo)	88·9	75 mg dm^{-3}	24 h	

be obtained by a sequenced anaerobic/aerobic treatment. Recently, however, the complete anaerobic mineralisation to methane of an azo dye decolourisation product (5-aminosalicylate) by a sludge adapted to the degradation of 2-nitro-phenol was reported.[21]

Primary azo-dye degradation has been generally proposed to be a non-enzymatic intracellular reaction in which reduced flavin nucleotides transfer electrons to the azo bond.[22] The fact that a higher degree of sulphonic acid substitution on several azo dyes greatly decreased their reduction rate was attributed to the inhibitory effect of this substitution on cell wall penetration. Moreover, all the dyes that were not reduced by whole cells could be reduced by cell extracts, indicating that dye permeation was a rate-limiting step in decolourisation. These reported decreased reduction rates could also be due to the more hydrophilic nature of these substituted dyes.[23] Contradictory results have however been reported as permeabilised cells showed a lower decolourisation capacity when compared with non-permeabilised cells[24] and it was suggested that dye degradation depends mostly on the reducing conditions provided by the bacteria, rather than on the interaction of the bacteria with the dye molecule.

A sequenced anaerobic/aerobic treatment can be obtained in a single reactor as aerobic and anaerobic microniches are easily established at the same time in aerated biofilms.[25] Two azo dyes (Acid Orange 10 and Acid Red 14) that were known not to be degraded in activated sludge systems were removed from a synthetic wastewater in an aerated biofilm reactor. The removal efficiencies were up to 60% at 5 mg dm^{-3}, but only if the dissolved oxygen level in the bulk liquid phase was below 1 mg O_2 dm^{-3}, and consequently with part of the biofilm under anaerobic conditions.[26] The same authors also reported decolourisation under highly aerated conditions (bulk dissolved oxygen around 6 mg O_2 dm^{-3}) but only of dyes known to be aerobically degradable (Acid Orange 8). Azo-reduction and partial mineralisation of azo dyes Mordant Yellow 3, Amaranth and Acid Red 1 could be achieved in aerobic conditions with calcium alginate entrapped cells of a *Sphingomonas* sp., due to the oxygen gradient established inside the immobilisation support.[27] Co-entrapment of this species with a 5-aminosalycilate-degrading strain led to complete mineralisation of Mordant Yellow 3.

A summary of laboratory work published on the subject of anaerobic dye decolourisation is presented in Table 2. Although distinct test conditions and dyes were applied some general observations concerning the factors that affect decolourisation can be distinguished.

2.2.2 *Toxicity/inhibition*

The anaerobic community that maintains the reducing conditions needed for the decolourisation of the textile dyes is known to be sensitive to toxic shocks and one of the reasons for the absence of colour removal in at least some of the reported experiments may be inhibition. Few data, however, have been reported on the inhibitory effect of the studied dyes on methanogenesis. Not only the dye itself can impair efficient colour removal but other compounds such as heavy metals, sulphide and salts are potentially inhibitory, as discussed in Section 2.5. The amines produced by the reduction of the azo bond are much less toxic to the methanogens than their parent compound.[38]

The successful decolourisation of various azo dyes in batch tests has been described[30] but one particular commercial azo-printing dye (Reactive Yellow 95) was not degraded. This was attributed to unidentified inhibitory compounds (impurities) in the dye solution. Other authors[19,35,36] reported reduced biodegradability for Reactive Blue 21 and Turquoise Blue G133 (two copper-containing dyes), which could be due to copper inhibition or to the fact that a non-azo chromophore was involved. Malpei and co-workers[40] found different toxic effects towards methanogenesis depending on the dye. The dyes with the lower inhibitory effects were found to be an azo reactive dye (Yellow Procion) and a cyanine reactive dye (Turquoise Cibacron). Methane yield was reduced 36·3% at 1500 mg dm^{-3} for Yellow Procion and 18·3% at 2400 mg dm^{-3} for Turquoise Cibacron. A possible explanation for the low inhibitory effect of the reactive dyes could be their high solubility and consequently low degree of interaction with the biomass. An interesting point as mentioned by the authors was the different behaviour with the two tested dispersed dyes. With an azo dispersed dye (Scarlet Resolin 3GL) a 60% inhibition of the methane yield occurred at 250 mg dm^{-3} but complete inhibition occurred at a concentration of 750 mg dm^{-3}. With the other tested dye (anthraquinone dispersed, Brilliant Red Resolin BLS) stronger inhibition occurred at the lowest tested concentration (78·9% inhibition at 300 mg dm^{-3}) but still at the highest tested concentration (2400 mg dm^{-3}) complete inhibition did not result. The authors explained this different behaviour by assuming that the different dyes inhibit different metabolic pathways. A reactor collapse occurred when a disperse dye (Disperse Blue 56, 30 mg dm^{-3}) was fed to a laboratory UASB, while the same biomass had shown good colour removal efficiencies with an acid dye (Acid Red 42, 80 mg dm^{-3}) and a direct dye (Direct Red 80, 40 mg dm^{-3}).[29] This was apparently due to the hydrophobic nature of the disperse dye, favouring its adsorption to the biomass and resulting in high (probably toxic) levels in the granules.

2.2.3 *Adaptation of decolourising microbes*

Adaptation is mentioned by several authors as an important feature for successful decolourisation. An increased resistance of an anaerobic consortium to the toxicity of a reactive azo dye was reported if the sludge

was previously exposed to the dye.[32] Other authors described the isolation of microbial consortia capable of a rapid anaerobic decolourisation of several commercially important dyes with the exception of a copper-containing phthalocyanine dye.[35,36] The same research group used this microbial consortium to inoculate a laboratory-scale upflow anaerobic filter, which attained high colour removal (>95%) with an initial dye concentration of 0·5 g dm^{-3}.[37] A decrease of the lag phase for the mineralisation of 5-aminosalicylate, an azo-dye metabolite, from 21 to 5 days after respectively 166 and 203 days of operation of a laboratory-scale UASB treating Mordant Orange 1 was described.[38] At a pilot plant, removal efficiencies for colour and COD were 10–20% higher if the reactors were inoculated with dye- and PVA-degrading bacteria.[41]

2.2.4 Co-substrates

Glucose, raw municipal wastewater and yeast extract, among others, have been reported as examples of an essential co-substrate needed to obtain good colour removal. Minimal concentrations of the co-substrate for good decolourisation are difficult to establish but the low colour removal capacities reported for some systems[31] could be due to the low organic load of the synthetic wastewater used. Several carbon sources have been compared[36] and glucose, glycerol and lactose gave the best results in relation to colour removal efficiency (82, 71 and 71%, respectively), while starch and distillery waste resulted in poorer decolourisation (52 and 39%, respectively). The successful adaptation to dye-metabolite degradation, as mentioned previously, occurred if glucose was used as co-substrate but could not be obtained when the acclimatisation occurred in the presence of a volatile fatty acids mixture (VFA).[38] However decolourisation of the original azo dye also proceeded with VFA as a carbon source, though less efficiently. The authors explained this difference in adaptation by a cross-feeding pattern. The establishment of a glucose-degrading consortium could possibly provide the 5-aminosalicylate-degrading bacteria with essential compounds such as vitamins or cofactors not present in the VFA-degrading consortia. The same research group reported that Azodisalicylate could be completely mineralised without the addition of an extra carbon source, but only after 423 days of acclimatisation of the anaerobic bacteria to Mordant Orange 1 and Azodisalicylate with glucose as a carbon source.[39]

2.2.5 Redox potential

The redox potential should reportedly be below −450 to −500 mV for azo dye reduction to occur.[32] If nitrate is present the redox potential is necessarily higher and no decolourisation is observed until complete nitrate removal.[22,32] The presence of sulphate as an additional electron acceptor has no apparent negative effect on dye reduction.[32] However, other researchers followed the redox potential in the anaerobic zone of a nutrient removal plant and concluded that values below −250 mV were sufficient to decolourise a reactive azo dye.[42] Kudlich *et al.*[43] found that quinone redox mediators with redox potentials between −137 and −225 were effective for the reduction of the azo dye Amaranth. These discrepancies point to the need for further research into this particular aspect.

2.3 Nutrients (nitrogen, phosphorus)

Dyebath additives containing nitrogen and phosphorus (e.g. urea, ammonium acetate, ammonium sulphate and phosphate buffers) are the main sources of nutrients in the textile effluent.[3] The mean value reported for a silk and lycra printing plant was 129 mg $NH_4^+ - N$ dm^{-3}.[44] A mixed textile (80%)–municipal (20%) effluent in Italy contained 89 mg $NH_4^+ - N$ dm^{-3}.[45] Nitrogen removal is commonly performed by nitrification/denitrification in anoxic/aerobic biological plants. Textile effluents were found to be major inhibitors of the nitrifying bacteria in aerobic treatment systems, thus hampering nitrogen removal.[46] The authors attributed this inhibition to chlorinated organic compounds and copper. It should be emphasised that anaerobic treatment can be effective in the removal of organic compounds, i.e. COD, but it is unable to remove mineralised compounds such as NH_4^+ and PO_4^-. Also, an external carbon source has to be available for biological denitrification in anoxic/aerobic arrangements. If the effluent in question has a too low COD/N ratio, nitrogen removal is inefficient. The addition of a mixture of methyl and ethyl alcohols to the above mentioned silk and lycra printing plant in order to increase the COD/N ratio was found to be essential for nitrogen removal.[44] Hence, in high-nitrogen waste waters it makes little sense to remove a part of the COD by anaerobic treatment in a first step when COD has to be added again to the effluent afterwards in order to achieve nitrogen removal. Paramount importance should be given in the COD/N ratio in considering anaerobic treatment of textile effluents. Effort should be put into the reduction of the amounts of dyebath additives applied and the selection of nitrogen- and phosphorus-free alternative auxiliaries in the production process, so as to minimise the need for nutrient removal in wastewater treatment.[2]

2.4 pH and salt effects

Acids and alkalies are used in the dyeing process depending on the dye class involved and large quantities of alkali are used in bleaching, desizing, scouring and mercerising. Extremes of pH have to be avoided in order to maintain good reactor performance in biological systems, thus making costly pH adjustment

necessary. The application of mineral acids for this purpose is also a source of salinity.[3] The use of acetic acid does not contribute to increased salinity but this component can account for 50–90% of the dyehouse organic load.[47] A high load of easily biodegradable compounds is not a constraint to the anaerobic process and the use of acetic acid in dyeing is thus beneficial from this viewpoint. It does however entail higher treatment costs if an aerobic process is applied. A buffer tank or the mixing of wastestreams from different processes can reduce pH fluctuations and minimise the need for pH adjustments. Advantage can be taken of the anaerobic process in this situation. The acidifying bacteria are more tolerant to pH fluctuations and can lower the pH of an alkaline influent as carbon dioxide and acids are produced. In a subsequent reactor optimum pH for the growth of the methanogens can be maintained. Acidification achieved by the injection of carbon dioxide is in this context an interesting option, as an accidental overdose cannot drop the pH below 6, no mineral salinity is introduced and there is no need to store large quantities of hazardous, concentrated acids.[48]

The inhibitory effect of salts on micro-organisms is mainly (but not only) related to the cations, of which the most common is sodium.[49] In laboratory-scale tests with flow-through anaerobic reactors it was found that reactors receiving a shock load of 30 g NaCl dm^{-3} were slightly inhibited, while a shockload of 35 g NaCl dm^{-3} reduced gas production by 65%.[50] A reactor gradually adapted to increasing amounts of salt was able to withstand salt concentrations of up to 65 g NaCl dm^{-3}. A 50% decrease in gas production was observed for the same reactor at 95 g NaCl dm^{-3}. Other authors[49] concluded that the safe operation of a UASB treating acidified wastewater is possible with salt concentrations up to 27–30 g NaCl dm^{-3}, while for unacidified wastewater the maximum tolerable concentration is 16–25 g NaCl dm^{-3}. No sludge adaptation to the sodium inhibition was observed upon 12 weeks exposure.

2.5 Inhibitory compounds

2.5.1 *General*

A wide range of compounds originating from the different textile manufacturing processes and contaminants from earlier, raw-material preparation processes will ultimately end up in the textile effluent, possibly hampering anaerobic treatment.[2,3] The same is also true for the aerobic process and is thus no reason to discard *a priori* the anaerobic option. High sludge retention times and short hydraulic retention times as encountered in UASB and biofilm reactors can be advantageous to increase biomass tolerance to inhibitory compounds as observed by Rinzema and co-workers for sodium.[49] Potentially problematic compounds are: biocides used in the growing or storage of cotton or wool (e.g. chlorinated aromatics), finishing products (e.g. synthetic resins), surfactants (e.g. alkyl phenol ethoxylates), dye solvents used for the dyeing of polyester fibres (e.g. trichlorobenzene, butylbenzoate), reducing agents (e.g. sodium sulphide, sodium hydrosulphite), heavy metals in metal complex dyes (e.g. copper) or used for dye fixation in wool dyeing (e.g. chromium), sulphate salts used as dyebath additives, oxidising agents (e.g. dichromate) and bleaching agents (e.g. hypochlorite, hydrogen peroxide). Dyes are potentially inhibitory, as discussed in Section 2.1.

The development of effective devices that protect the reactor from inhibition and/or the selection of micro-organisms which are able to withstand high levels of toxic compounds can in many cases prove to be essential tools for the successful application of anaerobic technologies in textile effluents. The PAD process (Primary Adsorption and sludge Digestion) is an approach to this problem.[51] It consists of a primary biosorption of colour and COD on anaerobic sludge. After adsorption and settling, digestion is used to regenerate the sorptive capacity of the biosorbent. This process was applied to treat an effluent from a carpet dyeing factory with a high organic load (3900–9300 mg COD dm^{-3}) mainly due to sizing agents and detergents. Colour removal in the sorption stage was up to 80% and COD removal up to 50%. Another way of overcoming toxicity could be upfront dilution.[52] In this technique the incoming wastewater is diluted to non-toxic levels with the effluent. In this way, the treatment of an industrial wastewater containing 10 g dm^{-3} formaldehyde and 20 g dm^{-3} methanol was possible with removal efficiencies of more than 98%. Other options include the adsorption of inhibitory components on activated carbon added to the reactor[53] or the use of a two-phase (pre-acidifiction + methanogenesis) anaerobic system.[54]

The most common potentially problematic compounds and their impact on treatment are considered in the following paragraphs.

2.5.2 *Sulphur compounds*

High sulphate levels (20–42 g dm^{-3}) can result in dyebath effluents if sodium sulphate is employed as an auxiliary in reactive dyeing.[55] In dyeing with sulphur or vat dyes sodium sulphide and sodium hydrosulphite are commonly used as reducing agents, ending up in the effluent. Another source of sulphur can be the use of sulphuric acid for pH control.[3] The EPA[1] mentioned for the various processing categories a maximum sulphide concentration of 8 mg dm^{-3} for wool finishing.

High concentrations of sulphate are considered to be undesirable as sulphate-reducing bacteria will compete with the methanogenic bacteria very efficiently for hydrogen and other substrates, and H_2S rather than CH_4 will be produced.[56] Hydrogen sulphide levels

above 200 mg dm^{-3} might inhibit methanogenic bacteria, although absolute inhibitory thresholds are difficult to establish as they generally result from the interaction of several factors. In the practical case mentioned in Section 3, exhausted reactive dyebath effluents were discharged at 6% (v/v) into a full-scale primary sludge digester, the sulphide concentrations increased but were consistently below (100 mg dm^{-3} and good reactor performance could be maintained.[55] On the other hand, the parallel laboratory-scale reactor fed with a three-fold higher loading rate collapsed due to the build-up of sulphide concentrations (up to 450 mg dm^{-3}). The authors had five suggestions to overcome the sulphur problem, namely, acclimatisation of the methanogens to higher sulphide levels, increasing the COD/SO_4^{2-} ratio, addition of metal salts in order to form sulphide precipitates and addition of molybdate in order to inhibit the sulphate-reducing bacteria. In the latter case no sulphide would be formed and sulphate is known to be much less inhibitory. The last option would be the substitution of sodium sulphate in the dyeing process by sodium chloride or sodium carbonate. In the case of sulphur dyeing sulphide can be replaced by glucose as a more environmentally-friendly reducing agent.[47] The resulting effluents could be handled in an anaerobic or aerobic treatment plant, although a higher carbonaceous organic load always point to the anaerobic option.

2.5.3 Heavy metals

The main source of heavy metals in the textile industry is the dyeing process. The maximum heavy metal contents of dyeing waste waters have been reported to be 12·1 mg Cu dm^{-3} for direct dyes on cotton, 2·7 mg Cr dm^{-3} for direct dyes on viscose, 7·5 mg Cd dm^{-3} for basic dyes on wool, and 3·4 mg Zn dm^{-3} for acid dyes on wool.[57] Most reported values for other dyes and fibres are below 1 mg dm^{-3}. For wool dyeing using optimised dyeing techniques the reported values are between 1 and 13 mg Cr dm^{-3}.[58] Many of the newly developed dyes are metal-free.[59,60] Literature on the toxicity of heavy metals on anaerobic digestion is extensive but general conclusions concerning the acceptable levels are difficult to establish because of the specific operating conditions employed in each case. For instance, a 50% inhibition of VFA degradation occurred by a Cr^{3+} concentration of 14·7 mg dm^{-3} in batch tests,[61] whilst other researchers mentioned that toxicity for the same compound gradually fed to a 5 dm^3 CSTR only occurred above 1140 mg dm^{-3}.[62] The major factors affecting inhibition include the type of compound concerned, exposure time, pH, temperature, sludge acclimatisation, presence of precipitating agents, hydraulic retention time, sludge retention time and sludge concentration.[61–63] Sulphide salts of most heavy metals, except chromium, form insoluble precipitates with reduced inhibitory effect. Cr^{6+} is reduced to Cr^{3+} which is poorly soluble at neutral pH.[56] Malina[56] stated that 1·8–2·0 mg dm^{-3} of heavy metals are precipitated as metal sulphides by 1·0 mg dm^{-3} of S^{2-}. High concentrations of sulphur are often encountered in textile effluents and thus can have a beneficial effect with respect to removal of toxicity caused by heavy metals.

2.5.4 Bleaching chemicals

Bleaching of textiles is currently carried out using hydrogen peroxide (H_2O_2) or chlorite. There is a trend in reducing the use of chlorite as carcinogenic adsorbable organic halogens (AOX) are formed.[2]

High levels of chlorite or hydrogen peroxide will cause inhibition problems in biological treatment processes.[3] Grüttner[46] attributed nitrification inhibition in aerobic treatment systems treating textile waste waters to AOX (1·7 mg dm^{-3} maximum concentration) and copper (0·9 mg dm^{-3} maximum concentration). Under anaerobic conditions reductive dehalogenation of the AOX can occur. Several laboratory- and pilot-scale trials have shown that higher removal efficiencies can be achieved with anaerobic systems (40–45% AOX removal) than with aerobic systems (30–35% AOX removal) at initial AOX concentrations up to 100 mg dm^{-3}. A sequenced anaerobic/aerobic configuration reached up to 50–55% AOX removal.[64] In order to protect a UASB treating a peroxide-containing pulp and paper mill effluent a pre-acidification tank was installed. This design allowed complete removal of up to 1200 mg H_2O_2 dm^{-3} without any detrimental effect noted on the anaerobic plant.[54]

2.5.5 Surfactants

A wide range of surfactants is currently employed in the wet textile operations to improve the wettability of the fibres. The recalcitrance to complete mineralisation of some products is cumbersome as they can cause foaming and toxicity problems in the receiving waterways. Selection of easily biodegradable surfactants is thus a step in the reduction of the detrimental effects of the plant effluents.[2,65]

The concentration of anionic surfactants in a silk and lycra printing plant was reported to be 13–14 mg dm^{-3}.[44] Wool scouring effluents can contain levels up to 800 mg dm^{-3} alkyl phenol ethoxylate (APEO).[66] Frassinetti *et al.*[67] mentioned concentrations for the recalcitrant APEO in textile effluents of up to 20–50 mg dm^{-3} and removal (not always complete mineralisation) efficiencies higher than 80% with conventional aerobic biological plants. However, surfactants in high concentrations will cause excessive foaming in aerobic treatment and lead to sludge bulking problems.[45]

Wagener and Schink[68] examined the possibility of treating a wastewater containing high levels of surfactants in a laboratory-scale anaerobic fixed-bed

reactor. They reported complete degradation of an alkyl ethoxylate (AE) with concentrations up to 1·0 mg dm^{-3}. Also primary degradation of APEO at an initial concentration of 500 mg dm^{-3} to polyethylene glycol (PEG) and alkylphenol was achieved. Further degradation of the latter compound did not take place. However, PEG was successfully converted to methane and carbon dioxide. Batch tests with anionic surfactants were not very successful. Alkylsulphonates and alkylbenzenesulphonates (ABS) were not degraded and inhibited anaerobic sludge at concentrations above 10 mg dm^{-3}. Primary degradation of sodium dodecylsulphate was achieved at concentrations up to 100 mg dm^{-3}.

Anaerobic degradation of AE and alkylsulphate in batch tests with anaerobic digester sludge was reported at concentrations of 10 mg dm^{-3} [69,70] and 1 mg dm^{-3}, respectively.[69] The mineralisation of AE was also reported in anaerobic pond sediments. ABS and stearyltrimethylammonium chloride however were not degraded even in sediments that had been exposed for over 25 years to high levels of these surfactants.[71]

3. PILOT- AND FULL-SCALE APPLICATIONS

A full-scale anaerobic plant with COD removal of 80% is in operation at a Spanish wool combing plant.[72] The effluent is then submitted to distillation. This scheme allows full recycling of the scouring water. A sequential anaerobic/aerobic full-scale plant is operating at a major dyehouse in Hong Kong.[73] An experimental anaerobic/aerobic treatment plant of a polyester and cotton factory was found to be successful.[74] The effluent was principally composed of dyes, auxiliaries, PVA, detergents, acids and alkali salts with COD levels of 600–900 mg dm^{-3}. The mean efficiencies for COD and colour removal were 78% and 72% respectively, with no reported toxicity. The anaerobic stage consisted of a rotating fixed film reactor and the loading rate varied between 1·66 and 2·71 kg COD m^{-3} day^{-1}. Good colour removal (80%) and COD removal (92%) were reported for the treatment of a cotton processing effluent in an anaerobic/aerobic pilot plant followed by activated carbon as tertiary treatment.[41] Fifty per cent of the colour removal was achieved in the anaerobic stage. The pilot plant was run for 10 months with an average load of 2·13 kg COD m^{-3} day^{-1} for the anaerobic biofilter and 1·71 kg COD m^{-3} day^{-1} for the aerobic biofilter. Inoculation of the reactors with enrichment cultures of dye-degrading and PVA-degrading bacteria was found to be an important factor in order to obtain successful operation.

An interesting approach to the colour problem was presented in South-Africa.[55] Exhausted reactive dyebath effluents were discharged at 6% (volume of dyebath/volume of influent sludge) into a full-scale digester treating primary domestic sludge during a 5-month test period. Colour was completely removed from the overflow and good reactor performance was maintained despite an increase in sodium and sulphide in the overflow. Similar tests were performed at laboratory-scale with a three-fold higher dyebath loading rate and no visible colour increase was observed. Nevertheless, stable performance could not be maintained, due to sulphide inhibition.

In a similar approach, the co-disposal of print pastes in anaerobic domestic sludge digesters was evaluated as a full scale alternative to incineration or landfilling.[75] In this study, laboratory-scale digesters were fed with primary sludge supplemented with 5% (on a weight basis) of a mixture of several print pastes, which represented 12% of the influent COD. Only after 3 months of adaptation was stable operation achieved. Good colour removal was observed in the supernatant and 75% of the organic load associated with the printing pastes was removed. The total nitrogen content of the paste was unchanged by the treatment, but the fraction present as ammonium increased, which was considered to be beneficial as ammonification of recalcitrant organic compounds is a constraint in biological nitrogen removal.

4 CONCLUSIONS AND PERSPECTIVES

Table 3 summarises the main factors, as discussed in the previous paragraphs, which can affect the decision to include an anaerobic stage in a textile wastewater treatment process. It is apparent that this anaerobic stage requires aerobic post-treatment in order to complete the mineralisation of some pollutants (dyes, surfactants, residual BOD) and remove nutrients. On the other hand, this aerobic post-stage benefits from the anaerobic pretreatment in several ways, including the protection against BOD and toxic shock loads, the possibility of reductive decolourisation and dechlorination, the minimisation of foaming and bulking problems and the reduction of aeration and sludge disposal costs.

Environmental concerns in the textile industry are presently inducing significant changes in the manufacturing processes. Limited availability of high-quality water supplies is forcing the implementation of in-process water-saving measures and advanced wastewater treatment for water recycling. This situation tends to generate concentrated wastestreams but, on the other hand, demands effluents with increased treatability. The latter is also promoted by the increasingly stringent discharge limits imposed by national and international legislations. The recycling of process chemicals is fairly established for synthetic sizes and mercerising baths and is now also being tested for some dyes. Another trend is the avoidance of process chemicals which are poorly

TABLE 3
Selection of Factors Affecting the Option for the Inclusion of Anaerobic Treatment in the Biotreatment of Textile Effluents

In favour	High BOD levels can be efficiently and cheaply removed
	Dyes can be reductively decolourised
	Heavy metals retained through sulphate reduction
	No foaming problems with surfactants
	High effluent temperatures can be favourable
	High pH effluents can be acidified
	Degradation of refractory organics can be initiated (e.g. surfactants, chlorinated aromatics)
Contrary	BOD removal can be insufficient
	Dyes and other refractory organics are not mineralised
	Nutrients (N, P) are not removed
	Sulphates give rise to sulphide
Not conclusive	Relative sensitivity of anaerobic and aerobic consortia, particularly if nitrification is required, to toxic/inhibitory components and to salinity is not established

biodegradable, toxic or inhibitory for microbial populations, or produce high salinity, high nutrient (N,P) levels or extreme pH values in the waste waters. However, when it comes to dyes, the tendency is for increased resistance to oxidising washing aids, possibly impairing the efficiency of removal processes based on ozonation or peroxidation.

On this line of evolution, anaerobic processes, with their capacity for efficient treatment of high-BOD, low-nutrient waste waters, and with their possibility of reductive dye decolourisation, become one of the most promising technologies for integrated wastewater treatment in the textile industry.

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Review

Colour in textile effluents – sources, measurement, discharge consents and simulation: a review

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Abstract: This paper aims to review the problem of colour in textile effluents, the different classes of dyes available and their contribution to the problem. Through new regulations, pressure is being placed on water companies all over the world to reduce the amount of colour in sewage effluent. Dyes exhibit low toxicity to mammals and aquatic organisms and therefore colour consents are normally applied for aesthetic and industrial reasons rather than for prevention of toxicity. The absorbance, ADMI values and concentrations of dyes in effluent are examined here with particular reference to reactive azo dyes used in cotton processing. Colour consents, the problem of colour in textile wastewaters and the importance for research in this area are also discussed. Dye concentrations of 0.01 g dm^{-3} up to 0.25 g dm^{-3} have been cited as being present in dyehouse effluent, depending on the dyes and processes used. ADMI values ranged from 50 to 3890 units for the dyeing of cotton. It was concluded that 1500 ADMI units was a reasonable value to aim for when simulating coloured effluents. Simulated textile effluents may be used for research purposes. These should resemble real wastes as closely as possible, but it is often difficult to replicate the ADMI values, absorbance and spectra of real effluents. The concentrations of dye used in simulated effluents examined in literature varied from 0.01 g dm^{-3} to 7 g dm^{-3}. As absorbance and ADMI values change with the types of dye used, it is difficult to relate these values to dye concentrations. A concentration of 0.18 g dm^{-3} of a Red or Yellow dye or 0.43 g dm^{-3} of a blue dye would provide an ADMI of approximately 1500 units and fits within the range of dye concentrations presented in literature. A dye mixture simulating colour in a real textile effluent is suggested and some limitations of simulating actual wastewaters discussed.

Keywords: colour; absorbance; ADMI; textile effluent; dye; consents

NOTATION

ADMI	American Dye Manufacturers' Institute
APHA	American Public Health Association
CIE	Commission International de l'Eclairage
CI	Colour Index
EA	Environment Agency
EQS	Environmental Quality Standard
LC_{50}	Lethal Concentration (50%)
NRA	National Rivers Authority
STE	Simulated Textile Effluent
STW	Sewage Treatment Works
TOC	Total Organic Carbon
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids

INTRODUCTION

Different dyestuffs have highly varying chemical characteristics and are selected according to the material to be dyed. Therefore the composition of dyeing effluent varies with the textile produced. Dyes are continually being upgraded and replaced by superior compounds. There is pressure on dye manufacturers to develop dyes that can be successfully applied using less auxiliary chemicals, especially salt, to reduce environmental problems associated with textile industry effluent. A single dyeing operation can use a number of dyes from different chemical classes resulting in a very mixed wastewater.[1] In 1978 it was estimated that 2%, or 9000 tonnes, of the 450 000

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Table 1. Percentage distribution of each chemical class between major application ranges (after Shore[15]) by permission of the Society of Dyers and Colourists

	% distribution between application ranges								
Chemical class	*Acid*	*Basic*	*Direct*	*Disperse*	*Mordant*	*Pigment*	*Reactive*	*Solvent*	*Vat*
Acridine		92		4				4	
Aminoketone	11			40	8		3	8	30
Anthraquinone	15	2		25	3	4	6	9	36
Azine	39	39				3		19	
Formazan	70						30		
Indigoid	2					17			81
Metal-complex azo	65		10				12	13	
Methine		71		23		1		5	
Nitro, nitroso	31	2		48	2	5		12	
Oxazine		22	17	2	40	9	10		
Phthalocyanine	14	4	8		4	9	43	15	3
Quinophthalone	30	20		40				10	
Stilbene			98					2	
Thiazine		55			10			10	25
Thiazole		5	95						
Triarylmethane	35	22	1	1	24	5		12	
Unmetallised azo	20	5	30	12	12	6	10	5	
Xanthene	33	16			9	2	2	38	

tonnes of dye produced world-wide were discharged in effluent from manufacturing operations while about 40000 tonnes, or 9%, was discharged in effluents from the coloration industries, giving a total of almost 50000 tonnes.[2] Collishaw *et al*[3] cited reports that the average world-wide growth in fibre consumption to the year 2000 would be from 2.2 to 2.6% p.a. with cotton consumption rising to 23 million tonnes. It can be assumed that dye consumption would rise concurrently with the increase in cotton consumption, and thus these figures give some impression of the scale of colour discharge.

Fashion has a role to play in determining the nature of textile effluent as it affects the fabrics and colours used and hence the dye types. Recently a fashion for black-dyed jeans resulted in the production of a highly coloured effluent in a Belgian factory which was very difficult to treat. In the East and North Midlands of the UK the colour of dye effluent was a problem historically, but the problem eased in the early 1980s due to changes in dye use and recession in the textile industry. By the late 1980s, however, revival of the industry meant the problem resurfaced, exacerbated by the popularity of cotton and use of reactive dyes.[4]

Technical and economic factors are also important in determining dye types used in the textile industry. Modern textile dyes are required to have a high degree of chemical and photolytic stability in order that they maintain their structure and colour.[5] They are designed to resist breakdown attributable to time and exposure to sunlight, water, soap, and other parameters such as bleach and perspiration. Anti-microbial agents are frequently used to make textiles, particularly natural fibres such as cotton, resistant to biological degradation.[6] The colour fastness, stability[7] and resistance of dyes to degradation have made colour removal from textile wastewaters difficult[8] as they are not readily degraded under the aerobic conditions prevailing in biological treatment plants[5,9] and therefore the effluents are in most cases coloured upon leaving the plant.[10] The dyes may not adversely affect the environment to which they are discharged but are of concern when the treated water is used as a supply of drinking water[10] or for other purposes. If water is coloured its uses can be limited and the aesthetics of the situation can lead to many complaints and possible breaches of discharge limits. Therefore there has been much research into colour removal from textile effluents, both using complex real effluents and more well characterised simulated wastes which are also useful for obtaining information on how individual dyes react to different types of treatment.

The primary aims of this paper are to review the problem of colour in textile effluents, the different classes of dyes available and their contribution to the problem. The absorbance, ADMI values and concentrations of dyes in effluent are examined with particular reference to reactive azo dyes used in cotton production. Colour consents, the problem of colour in textile wastewaters and the importance for research in this area are also discussed. A review of some of the Simulated Textile Effluents (STEs) available is presented, as this paper aims to advise on dye concentrations in their use. A simple dye combination for simulated cotton effluent is suggested which is representative of colour in real effluents. The limitations of simulated effluents are discussed.

DYES AND DYEING

One well-known system of classification internationally used for dyes is the Colour Index, devised by the

Table 2. Application classes of dyes and their chemical type (after Kirk-Othmer,[11] copyright © John Wiley & Sons Inc, 1993, reprinted by permission of John Wiley & Sons Inc)

Class	*Substrates*	*Method of application*	*Chemical types*
Acid	Nylon, wool, silk, paper, inks, and leather	Usually from neutral to acidic dyebaths	Azo including premetallised anthraquinone, triphenylmethane, azine, xanthene, nitro, and nitroso
Azoic components and compositions	Cotton, rayon,[a] cellulose acetate,[b] and polyester[b]	Fibre impregnated with coupling component and treated with a solution of stabilised diazonium salt	Azo
Basic	Paper, polyacrylonitrile-modified nylon,[c] polyester, and inks	Applied from acidic dyebaths	Diazacarbocyanine, cyanine, hemicyanine, diazahemicyanine, diphenylmethane, triarylmethane, azo, azine, xanthene, acridine, oxazine, and anthraquinone
Direct	Cotton, rayon,[a] paper, leather, and nylon	Applied from neutral or slightly alkaline baths containing additional electrolyte	Azo, phthalocyanine, stilbene, and oxazine
Disperse	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions often applied by high temperature–pressure or lower temperature carrier methods; dye may be padded on cloth and baked on or thermofixed	Azo, anthraquinone, styryl, nitro, and benzodifuranone
Fluorescent brighteners	Soaps and detergents, all fibres, oils, paints, and plastics	From solution, dispersion, or suspension in a mass	Stilbene, pyrazoles, coumarin, and naphthalimides
Food, drug and cosmetic	Foods, drugs, and cosmetics		Azo, anthraquinone, carotenoid, and triarylmethane
Mordant	Wool, leather, and anodised aluminium	Applied in conjunction with chelating Cr salts	Azo and anthraquinone
Natural	Food	Applied as mordant, vat, solvent, or direct and acid dyes	Anthraquinone, flavonols, flavones, indigoids, chroman
Oxidation bases	Hair, fur, and cotton	Aromatic amines and phenols oxidised on the substrate	Aniline black and indeterminate structures
Pigments	Paints, inks, plastics, and textiles	Printing on the fibre with resin binder or dispersion in the mass	Azo, basic, phthalocyanine, quinacridone, and indigoid
Reactive	Cotton, wool, silk, and nylon	Reactive site on dye reacts with functional group on fibre to bind dye covalently under influence of heat and pH (alkaline)	Azo, anthraquinone, phthalocyanine, formazan, oxazine, and basic
Solvent	Plastics, gasoline, varnish, lacquer, stains, inks, fats, oils, and waxes	Dissolution in the substrate	Azo, triphenylmethane, anthraquinone, and phthalocyanine
Sulfur	Cotton and rayon[a]	Aromatic substrate vatted with sodium sulfide and re-oxidised to insoluble sulfur-containing products on fibre	Indeterminate structures
Vat	Cotton, rayon,[a] and wool	Water-insoluble dyes solubilised by reducing with sodium hydrosulfite, then exhausted on fibre and re-oxidised	Anthraquinone (including polycyclic quinones), and indigoids

[a] Rayon now referred to as viscose.
[b] Azoics no longer used on polyester and cellulose acetate.
[c] Should read basic-dyeable nylon.

Society of Dyers and Colourists in 1924. This classifies dyes by firstly assigning each a generic name determined by its application characteristics, and then assigning a CI constitution number based on its chemical structure if known.[5] Companies need to register (free of charge) with the Society of Dyers and Colourists if they wish to use CI numbers and generic names. Dyes can be classified by their chemical structure or application method.[11] The percentage distribution of dye classes between major application ranges can be seen in Table 1. The dye classes, substrates, method of application and chemical types

Dye application class	Fibre	Degree of fixation (%)	Loss to effluent (%)
Acid	Polyamide	89–95	5–20
Basic	Acrylic	95–100	0–5
Direct	Cellulose	70–95	5–30
Disperse	Polyester	90–100	0–10
Metal-complex	Wool	90–98	2–10
Reactive	Cellulose	50–90	10–50
Sulfur	Cellulose	60–90	10–40
Vat	Cellulose	80–95	5–20

Table 3. Estimated degree of fixation for different dye-fibre combinations and loss to effluent (after Easton[5]) by permission of the Society of Dyers and Colourists

can be seen in Table 2. Sulfur dyes are not presented in Table 1 as their structures are undetermined and they therefore do not fit into any given chemical class. A description of the dyes, their characteristics and applications can be found in the literature.[12] In recent years some azo dyes have been banned by the German government for use with textiles for body-contact end-uses as they produce carcinogenic amines on degradation. However, many acid and direct dyes which may liberate amines such as benzidine, *o*-tolidine and *o*-dianisidine are still used.[13] In 1986 the Health and Safety Executive in the UK issued publications on potential respiratory irritant and sensitisation effects caused by handling of dye powders or exposure to their aerosols.[14] The use of dyes, as most chemicals, can be hazardous when used without the required precautions and safety measures.

In 1990 Shore cited that there were over 2000 entries in the colour index for acid dyes, 55% of which were still active (in use). The next most common were direct dyes (~1450 entries, 40% active) and disperse dyes (~1250 entries, 60% active). The use of reactive dyes (75% active), solvent dyes (60% active) and pigments (60% active) is increasing, but the use of vat dyes (45% active) and mordant dyes (33% active) appears to be declining.[15] The rapid growth in the use of reactive dyes is due to the increasing use of cellulosic fibres[16] and the technical and economic limitations of other dyes used for these fibres. If colorant precursors and sulfur dyes of indeterminate constitution are excluded, then two-thirds of the organic colorants listed in the Colour Index are azo dyes, with one-sixth of them being metal complexes.[5] Only about 30 of the approximately 3000 chemical compounds listed in the Colour Index are used at rates in excess of 1000 tonnes per annum. These comprise about 15% of the tonnage. Ninety per cent of the products are used at the rate of 100 tonnes per annum or less. Eighty per cent of textile dye users purchase 200 kg or less of dye per annum.[17]

The degree of fixation for different dye and fibre combinations can be seen in Table 3. Some of the figures for loss of dye to effluent differ slightly from the earlier estimates of Laing (1991) who gave the loss of Basic Dyes as 2–3%, Direct 5–20%, Disperse 1–20%, Metal-complex 2–5%, Sulfur 30–40%, and Reactive 20–50%.[17] It is seen that reactive dyes have rather low rates of fixation while the highest fixation rates are achieved with basic dyes. After the reactive dyeing process is complete up to 800 mg dm^{-3} of hydrolysed dye may remain in the bath.[18] Efforts are being made to increase the rate of fixation of reactive dyes by reducing the quantity of dye hydrolysed[19] which accounts for the range of variation in fixation. Fixation rates for reactive dyes tend to be in the range of 60–70%[19] although fixation rates tend to be higher in dyes containing two reactive groups.[20] Therefore up to 40% of the colour is discharged in the effluent, resulting in high colour of effluent from reactive dyeing operations. An additional problem is that reactive dyes in both the ordinary and hydrolysed forms are not easily biodegradable and thus even after extensive treatment colour from unexhausted reactive dyes may still remain in textile wastewater.[21,22] The cotton industry is therefore strongly associated with coloured effluent. Cotton effluent compares poorly in terms of colour to effluent from, for example, the wool industry where up to 98% of the dye applied is absorbed by the wool, resulting in effluent that is far less coloured (Table 3).

The popularity of different dye types in the textile industry can be seen by examining the percentage of each class in wastewater from two catchments in the UK (Table 4). This table emphasises the importance of treatment of cotton effluent as reactive dyes are dominant in both catchments due to the current trend for use of cotton fabrics. Disperse dyes, direct dyes and sulfur dyes are also popular. The use of dye types varies with catchment, presumably attributable to the

Table 4. Percentage use of dyes by class in two catchments in the Severn Trent water treatment catchment area (adapted from Churchley[23] with permission from Elsevier Science)

Dye class	Loughborough catchment	Wanlip catchment
Reactive	60.8	66.7
Metal	2	4
Disperse	14.9	7.2
Basic	1	6
Acid	5	4.8
Sulfur	4.2	9
Direct	11.4	1.5
Mordant	1	0

types of textiles processed in each area. It can be seen that of all dye classes reactive dyes present the greatest problem in terms of colour, which is exacerbated by the dominance of cotton in today's fashion industry.

COLOUR DISCHARGE CONSENTS

Dyes exhibit low toxicity to mammals and aquatic organisms.[24] This was demonstrated by Clarke and Anliker[25] who surveyed about 3000 colorants and found only 2% of the dyes had an LC_{50} to fish of $<1.0\,mg\,dm^{-3}$ and over 96% of dyes had an LC_{50} above $10\,mg\,dm^{-3}$. The eye can detect concentrations of $0.005\,mg\,dm^{-3}$ of reactive dye in water[26–28] and therefore concentrations of dye exceeding this would not be permitted on aesthetic grounds. Hence consent levels for the discharge of colour to receiving waters are normally applied for aesthetic reasons and not for prevention of toxicity. Diaper *et al*[29] reported that from the literature there appeared to be little evidence for bioaccumulation of the more commonly used dyes.

Rivers in the UK Midlands have improved in quality since the 1950s due to a range of improvements in water treatment which has resulted in decreased turbidity of river water and hence increased visibility of coloured pollutants.[30] The fashion for brilliant hues in full depths on cotton has also resulted in an increase in the quantities of reactive dyes used.[23] Therefore since the mid 1970s there have been complaints about colour in some rivers in the UK Midlands.[30] Most complaints made concerning colour tend to refer to water containing red dyes.[31] Colour in textile effluents, particularly red hues, is usually linked to the presence of reactive azo dyes in the water.[32] There are far less complaints regarding colours such as blue, green or brown as these are colours that might be expected of rivers or are less noticeable. Up to 1995 colour of water accounted for about 5% of complaints received by the National Rivers Authority (NRA) of the UK, now superseded by the Environment Agency (EA).[9] In 1992 in the UK colour was the subject of more than 500 complaints, mostly from the Severn Trent region.[28] Through new regulations, pressure is being placed on water companies in Britain to reduce the amount of colour in sewage effluent. In 1989 the NRA announced their intention to tighten the consent conditions for Sewage Treatment Works (STW) in Seven Trent, including those for colour.[30] Most standards applied to water measure the pollutant in $mg\,dm^{-3}$. It is not feasible to express colour standards in this way, however, as similar concentrations of different dyes can produce totally different results both in terms of colour and intensity.[9] Therefore absorbance and, less commonly, ADMI values are used to express colour limitations on discharge.

In the UK the EA determines what may and may not be discharged to river water. In setting consents for discharges, the EA assesses the proposed concentration of the parameter under consideration against an Environmental Quality Standard (EQS) which shows the concentration which should not be exceeded downstream of the discharge. Mass balance calculations are carried out to determine the concentration of the parameter which can be discharged without exceeding the EQS. To date there is no EQS for colour, however, and therefore the practice is to determine an acceptable colour for the river to which the effluent is to be discharged, usually by taking samples of the river water and assessing which samples are acceptable to the eye. The absorbance of the samples at a range of wavelengths is then measured and the results used as a basis for the determination of consent limits. Consent limits are usually expressed as absolute limits for the wavelength within a particular range. Samples are filtered through a 0.45 μm filter and absorbance in a 1 cm cell measured between 400 and 700 nm. Standards are usually expressed at 50 nm intervals although exceptions may be made if significant intermediate peaks are present.[9] In general there are unlikely to be narrow peaks present across the wavelengths, however, as factories tend to produce effluent containing a wide range of dyes.[33] The colour standard for the river must be met at times of low flow conditions upstream when the river upstream is of normal colour and average daily effluent flows are being discharged to the river.

The permitted colour of the discharge is calculated as follows:

$$C_d = \frac{[(C_s \times F_t) - (C_u \times F_u)]}{F_d}$$

where:

C_d = colour limit for discharge (absorbance cm^{-1})
C_s = colour standard for the watercourse (absorbance cm^{-1})
C_u = natural colour of watercourse upstream of discharge (absorbance cm^{-1})
F_d = mean discharge flow ($Mdm^3\ day^{-1}$)
F_t = total river flow downstream of discharge ($Mdm^3 day-1$)
F_u = 95% exceedence river flow upstream of discharge ($Mdm^3\ day^{-1}$)

(Environment Agency, Cardiff, UK *pers comm* (1998))

Some colour consent values for STWs receiving coloured trade effluents can be seen in Table 5. The absorbance of samples is read after filtration through a 0.45 μm membrane filter in a cell with 10 mm path length against a blank of demineralised water. It can be seen that the colour consents for Leek, Wanlip and Pinxton are all lower than the typical colour consent provided by the EA. This is due to the fact that there are many textile works in the Severn Trent area and so in order to prevent pollution of the receiving waters each sewage works is more limited as to the quantity of colour they may discharge to the rivers. The 'typical' colour consent comes from a region where there are not many dyeworks in the locality which explains why it is so much higher than those for the other works.

Colour wavelength (nm)	Leek	Wanlip	Pinxton	Typical colour consent[a]
400	0.060	–	–	0.115
450	0.040	–	–	0.085
500	0.035	0.020	0.028	0.065
550	0.025	0.021	0.025	0.055
600	0.025	0.012	0.024	0.040
650	0.015	0.012	0.017	0.028
700	–	–	–	0.013

[a] This information was supplied by the Environment Agency (Shrewsbury, UK pers comm (1998)).

Table 5. Colour consent standards for STW receiving coloured trade effluents in three sewage works in the Severn Trent area (after Upton and Churchley[30]) and 'typical' consent values (EA)

The ADMI (American Dye Manufacturers' Institute) measurement can be used for determining the colour of samples. The method involves measuring the absorbance of samples, after filtration through filter gel,[34] at sets of 10 or 30 wavelengths depending on the accuracy required to generate the CIE (Commission International de l'Eclairage) Tristimulus Values, X, Y and Z. These are converted by the use of published tables to values called Munsell values. From the Munsell values the Adams–Nickerson Colour Difference (DE) is calculated from an equation. The DE values of a series of APHA platinum–cobalt standards is plotted against the corresponding ADMI values to give a calibration plot and the DE value of samples read against this plot to obtain the ADMI value of the sample. The measurement is carried out both at the normal pH of the sample and at pH 7.6.[34]

DYE CONCENTRATIONS IN SIMULATED AND REAL TEXTILE EFFLUENTS

Textile wastewater treatment may require the removal of over 99% of the colour in samples and for this level of colour removal to be maintained with large volumes of rapidly changing effluents.[35] Research into treatment of coloured effluents is therefore very important and there has been much research into different treatment methods.

Many researchers have used artificial textile wastes (Table 6) in the investigation of treatment technologies. This is useful in two ways: firstly it enables research to be carried out in the absence of a local source of effluent; secondly, simulated effluents have constant composition and therefore enable the effects of treatment to be more readily understood. It can be seen that the concentrations of dye used varied from $0.01\,g\,dm^{-5}$ to $7\,g\,dm^{-3}$. This variation can be attributed to a number of factors: firstly, it is difficult to find any figures relating to actual concentrations of dyes in real wastes as most authors discuss colour in terms of absorbance or ADMI values. Secondly, the quantity of dye in the effluent varies with the dye type due to different exhaustion rates. The types and range of processes used can also affect the composition of the final effluent. The description of textile effluent in terms of absorbance and ADMI is more useful in terms of describing a pollutant than is dye concentration, as different dyes give rise to different intensities and colours. It does make it difficult to determine a useful dye concentration for simulated wastes, however. As absorbance and ADMI change with the types of dye used, there is not a direct relationship between these values and dye concentrations.

There are few reports of measured dye levels in rivers.[5] However, Laing[17] reports that normally dye concentrations of $0.01–0.05\,g\,dm^{-3}$ are present in

Author	Concentration ($g\,dm^{-3}$)	Type
Basibuyuk and Forster[36]	0.025, 0.05	Basic
Basibuyuk and Forster[37]	0.025, 0.2	Acid, basic
Carliell *et al*[38,32]	0.1; 0.1–0.2	Reactive
Carrière *et al*[39]	0.1, 0.5	Disazo acid
Chang *et al*[40]	0.01	Basic
Jia *et al*[41]	0.05	Reactive, acid, direct, sulfur, vat and others
Kace and Linford[42]	0.1	Disperse
Kang and Chang[43]	0.02	Reactive
Kirby *et al*[44]	0.5	Reactive, diazo, azo, phthalocyanine, disperse
Koprivanac *et al*[45,46]	7	Reactive
Li and Zhang[47]	0.1	Reactive, disperse, sulfur, direct, vat
Márquez and Costa[48]	0.02	Acid
Panswad and Wongchaisuwan[49]	0.3	Reactive
Wilcock *et al*[50]	2.5	Disperse
Yeh and Thomas[51]	0.025–0.2	Disperse
Zissi and Lyberatos[52]	0.010	Disperse

Table 6. Dye concentrations used by some authors in simulated textile effluents

Table 7. Absorbance of typical site effluent (after Hoyle[35] by permission of the Society of Dyers and Colourists), three PROCION dyes, and a mixture of the three dyes at wavelengths used for EA consent standards

Wavelength (nm)	*Typical site effluent*	*Red H-E7B (0.125 g dm^{-3})*	*Yellow P3R (0.125 g dm^{-3})*	*Blue P-GR (0.125 g dm^{-3})*	*Dye mixture (0.2 g dm^{-3} total)*
400	1.423	0.521	1.745	0.332	1.562
450	1.529	0.472	1.662	0.079	1.146
500	1.965	1.774	0.566	0.213	1.267
550	1.715	2.121	0.045	0.586	1.546
600	1.514	0.065	0.006	0.948	0.951
650	0.772	0.004	0.004	0.806	0.784
700	0.62	0.003	0.004	0.131	0.131
Mean	1.283	0.709	0.576	0.442	1.055

dyehouse effluent. Several authors report that a dyehouse dyeing cotton with reactive dyes would be expected to discharge 0.06 g dm^{-3} of dyestuff.[26,28] Other figures cited are 0.06 g dm^{-3} when reactive dye is applied by exhaustion in a jet dyeing machine, and approximately 0.25 g dm^{-3} when applied continuously using a pad mangle.[53] Gähr *et al*[54] give 0.1–0.2 g dm^{-3} as the normal range of reactive dyebath residues. Vandevivere *et al*[55] cite a range of 0.6–0.8 g dm^{-3}. It can be seen that most dye concentrations cited for both real and simulated wastes are below 0.5 g dm^{-3}. However, Koprivanac *et al*[45,46] stated that 7 g dm^{-3} dye was representative of concentrations found in waste streams in the reactive dye industry when discussing the choice of simulated textile wastewater. This greatly exceeds the concentrations mentioned by other authors and may be representative either of a particular factory or of very concentrated effluent. If this value is excluded then it can be seen that the range is far narrower.

Ghorpade and Spencer[56] give typical intensities of colour in dye wastes as 1000–1500 ADMI units. Goronszy and Tomas[57] noted that the effluent to be treated from a Puerto Rican plant dyeing cotton and 50% polyester/cotton had a colour in the range of 50–2000 ADMI units over a 4 month period. Correia *et al*[1] cited ADMI values ranging from below 50 for dyeing water from continuous disperse/acid/basic dyeing of nylon to 12500 for effluent from direct dyeing of viscose. They cited an ADMI of 1390 units for reactive dye wastewater from a continuous process and 3890 for reactive dye wastewater from a batch process. Therefore it seems that 1500 ADMI units is a reasonable value to aim for when simulating cotton effluents.

Three PROCION dyes with known structures, Yellow P3R (CI Reactive Orange 12), Red H-E7B (CI Reactive Red 141) and Blue P-GR (CI Reactive Blue 5), were tested at concentrations of 0.075, 0.075 and 0.15 g dm^{-3} respectively to determine their ADMI values. These concentrations were selected to permit good transmittance values to be obtained. The amount of pure colour present in reactive dyes as supplied is typically in the range of 25–60%, the remainder being comprised of substances such as salt, diluents such as sodium lignosulfonate, moisture and small quantities of other dye colours. Samples were not filtered through gel as they had no turbidity, and were measured at pH 7.6 using the 30 co-ordinate method. ADMI values of the dyes were found to be 8107, 8320 and 3440 units per gram of each dye respectively. Hence solutions containing 0.18 g dm^{-3} of the yellow or red dye, or 0.43 g dm^{-3} of the blue dye would give an ADMI of approximately 1500 units. The concentrations of these dyes fit within the broad range of dye concentrations used by other authors when a 'typical' ADMI value is present in the water. These results show that different dyes give rise to different ADMI values and thus concentrations of dye in water cannot be calculated from the ADMI readings if more than one dye is present.

SIMULATING THE COLOUR OF TEXTILE EFFLUENTS

The generation of synthetic wastewaters of defined composition is necessary for research but there is little information available on simulating the colour of textile effluents. Frequently one dye is used in simulated wastes, as can be seen in many of the cases listed in Table 6. However, on occasion it is useful to study wastes that are more representative of real effluents in terms of colour. An attempt was made to imitate the spectrum of a real effluent with a simulated wastewater of simple composition. The dye concentrations required to simulate the spectrum of a real effluent were determined and the ADMI value was

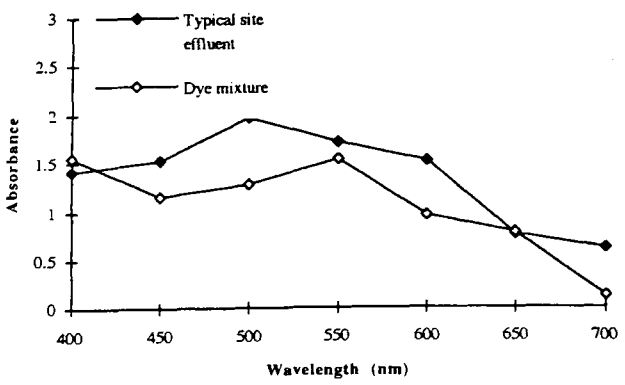

Figure 1. Absorption of the typical site effluent and dye mixture.

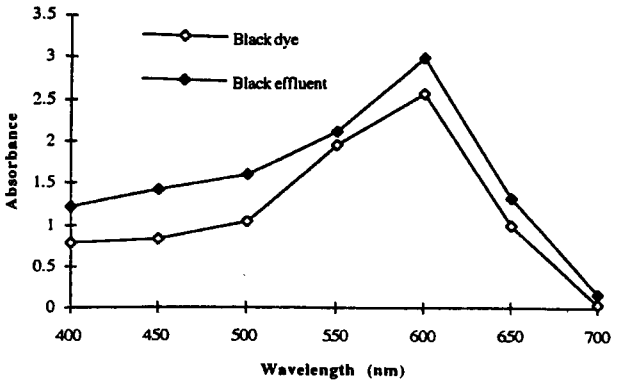

Figure 2. Absorption of black dye and black effluent from a jeans dyeing factory.

estimated for these concentrations. Other substances, such as starch or surfactants, could be added as required.

The absorbance of a 'typical dyehouse end-of-pipe general site effluent'[35] is seen in Table 7. The nature of the dyes present in the typical effluent in Table 7 is not specified but the spectrum was imitated using reactive dyes to generate a simulated cotton effluent. In order to do this the absorbance of three dyes with peaks at different parts of the spectrum was measured over the consent wavelengths in a 10 mm light path. The Yellow, Red and Blue reactive dyes discussed earlier were used. The Yellow dye peaked in the early part of the spectrum, the Red in the middle of the spectrum, and the Blue towards the end of the spectrum. The dyes were then mixed together at suitable concentrations in an attempt to imitate the spectrum of the typical site effluent and the absorbance of the resulting solution measured between 400 and 700 nm (Table 7). Concentrations used were 0.05 g dm^{-3} of the Red and Yellow dyes and 0.1 g dm^{-3} of the Blue dye.

A graph representing the absorbance of the typical site effluent in Table 7 and dye mixture as described in Table 7 can be seen in Fig 1. The absorbance of the simulated waste, using a combination of only three dyes, was similar to that of the real effluent. The total dye concentration required in the simulated waste for this purpose was 0.2 g dm^{-3}. Using the ADMI values of the dyes obtained previously, it was estimated that this would give a total of 1165 ADMI units. Both the dye concentration and the ADMI value were within the normal range of dye concentrations described earlier. This showed that the spectrum of real effluent can be reasonably simulated using a small number of dyes at concentrations and ADMI values which are within the normal limits. Kirby *et al*[44] generated a simulated textile effluent containing nine dyes, a total concentration of 0.5 g dm^{-3} dye, and achieved an absorbance of 2.5–3 units between 300 and 640 nm. This was higher than that of the 'typical site effluent'. However, textile effluent composition is extremely variable and therefore differences in absorbance between effluents is common. The spectrum of this simulated effluent had no sharp peaks or dips until 640 nm in accordance with Hazel.[33] Absorbance of the 'typical site effluent' also dropped after 600 nm.

If it was necessary to imitate any particular effluent, the absorbance would have to be examined at intervals of 50 nm as in Table 7. Appropriate dyes of different colours would then be selected to give peaks in appropriate parts of the spectrum and their concentrations altered to give peaks of the correct intensities. However, it has been reported that the spectrum of a mixture of dyes may differ from that predicted from the spectra of the individual dyes. This is principally observed with direct cotton dyes and may be caused by the formation of dye complexes in the aqueous phase.[58] This can add to the difficulty of attempting to imitate a spectrum.

It must be noted that imitating the spectrum alone of an industrial effluent will not necessarily provide accurate information on how the absorbance will be affected by treatment. In studies carried out by the authors, a solution of Remazol Black B dye was found to have a spectrum comparable to black effluent from a jeans dyeing factory (Fig 2) which used a dye of unspecified structure described as Black Black. Remazol Black B (20 mg dm^{-3}) was subjected to biotreatment in 1 dm^3 reactors operating in sequential batch mode (SBR). The reactors were inoculated with sludge taken from full-scale continuous activated sludge reactors receiving mixed domestic-industrial wastewater. They were then fed with 750 mg COD dm^{-3} hydrolysed sizing agent, and inorganic nutrients. The dyes were injected into the reactor once the fill phase was complete. The SBRs had a 24 h cycle of operation of which the first 13 h were anaerobic. The Oxidation Reduction Potential was −350/−400 mV (for a silver/silver chloride reference electrode) during this anaerobic phase. After 4 h of anaerobic biotreatment the black dye turned violet, exhibiting a spectrum similar to that of Remazol Brilliant Violet 5R, to which it is structurally related. Cleavage of one of the two azo bonds in the black dye would give a structure similar to that of the violet dye, with the exception of some substituents. However, when the violet dye (100 mg dm^{-3}) was subjected to similar anaerobic bio-treatment it was almost completely decolourised over the 13 h anaerobic period with 80% removal occurring after 4 h, while the violet colour from degradation of the black dye remained unchanged throughout the remainder of a 24 h experimental period. Therefore although the spectra of the violet dye and the black dye after 4 h of anaerobic treatment resembled each other, the two dyes demonstrated totally different biochemical behaviour.

Similar observations were made with PROCION Blue MX-R and Remazol Blue 19 which have similar spectra and structures which only differ in their reactive groups. Batch digestibility and inhibition tests (towards acetate–propionate-consuming bacteria) were carried out at 35 °C using anaerobic sludge (0.5 g VSS dm^{-3}) originally from a sewage treatment

works, maintained in the laboratory on a glucose medium. In the toxicity tests a VFA spike was added and its consumption measured for 6–8 days for comparison with a control without dye. The inhibition was measured as the cumulative VFA consumption (% of the control). In batch tests at 50 mg dm^{-3} dye, the first dye exhibited no inhibition and 66% colour removal at the wavelength of maximum absorption, while the second caused 85% inhibition but 97% colour removal was achieved. Again, dyes with similar spectra and structures showed different biochemical behaviour.

It is therefore apparent that aspects other than colour must be considered in order to obtain an effluent that behaves in a similar manner to real wastewater under treatment. Rozzi *et al*[59] investigated distribution of absorbance in the visible spectrum in relation to molecular size in secondary, intermediate and final effluent from a municipal plant treating textile wastewater. They found poor correlation between the distribution of percentage TOC and percentage aborbance in relation to molecular size. This indicated that spectral analysis was unsuitable for monitoring organic pollution in textile effluents as intensity of colour did not necessarily reflect the quantity of organic pollutants present. Therefore, given that colour alone does not reflect the degree to which effluent is polluted and the fact that dyes with similar spectra may behave in a biochemically different manner, the importance of imitating other characteristics, such as COD and toxicity, is highlighted in the generation of simulated textile effluents.

CONCLUSIONS

Literature cites dye concentrations below 1 g dm^{-3} as being typically present in dyehouse effluent, normally comprised of a large number of dyes. The concentrations of dye used in simulated effluents in the literature quoted here varied from 0.01 g dm^{-3} to 7 g dm^{-3}. Reported ADMI values for real effluents in the literature ranged from 50 to 23890 units. It is difficult to relate dye concentrations to colour measurements as dyes absorb differently in different parts of the spectrum. Therefore the colour of effluent is described in terms of absorbance at specified wavelengths or ADMI measurements.

Consent levels for the discharge of colour to receiving waters are normally applied for aesthetic reasons or industrial reasons as dyes exhibit low toxicity to mammals and aquatic organisms.

In composing a simulated textile effluent for research purposes, a concentration of 0.18 g dm^{-3} the yellow or red dyes used here, or 0.43 g dm^{-3} of the blue dye gave an ADMI value representative of those cited by other authors. A solution containing 0.05 g dm^{-3} of red, 0.05 g dm^{-3} yellow and 0.1 g dm^{-3} blue dye produced a spectrum similar to that of a typical site effluent.

While it is possible to imitate the spectrum of textile wastes adequately, dyes exhibiting similar spectra may behave very differently during treatment. Poor correlation exists between TOC distribution and percentage absorbance in relation to molecular size which means that colour of an effluent is not necessarily indicative of its level of pollution. Therefore imitation of the spectrum alone may not be sufficient in generation of simulated wastewaters for research purposes.

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SHORT CONTRIBUTION

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Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent

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Abstract Decolorisation of azo dyes during biological effluent treatment can involve both adsorption to cell biomass and degradation by azo-bond reduction during anaerobic digestion. Degradation is expected to form aromatic amines, which may be toxic and recalcitrant to anaerobic treatment but degradable aerobically. Methods for the quantitative detection of substituted aromatic amines arising from azo-dye cleavage are complex. A simple qualitative method is suggested as a way in which to investigate whether decolorisation is actually due to degradation, and whether the amines generated are successfully removed by aerobic treatment. Samples from a combined anaerobic-aerobic system used for treating a simulated textile wastewater containing the reactive azo dye Procion Red H-E7B were analysed by high-performance liquid chromatoraphy/ultraviolet (HPLC-UV) methods. Anaerobic treatment gave significant decolorisation, and respiration-inhibition tests showed that the anaerobic effluent had an increased toxicity, suggesting azo-dye degradation. The HPLC method showed that more polar, UV-absorbing compounds had been generated. Aerobically, these compounds were removed or converted to highly polar compounds, as shown by HPLC analysis. Since the total organic nitrogen (TON) decreased aerobically as organic N-containing compounds were mineralised, aromatic amine degradation is suggested. Although only a simple qualitative HPLC method was used, colour removal, toxicity and TON removal all support its usefulness in analysing biotreatment of azo dyes.

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Introduction

Many dyes used by the textile industry cannot be degraded, and hence decolorised, aerobically (Pagga and Brown 1986) as the enzymes involved aerobically are dye-specific (Zissi and Lyberatos 1996). Azo dyes may be decolorised by cleavage of the azo bond, with which the colour is associated, via anaerobic degradation through the action of non-specific enzymes (Boe et al. 1993; Zaoyan et al. 1992). These dyes are reduced and hence decolorised when acting as electron acceptors for the microbial electron transport chain, so a source of labile carbon is required (Carliell et al. 1996).

Cleavage of the azo bond generates aromatic amines (FitzGerald and Bishop 1995) which, with few exceptions (Razo-Flores et al. 1997), are not degraded anaerobically (Brown and Hamburger 1987). Dyes are not normally cytotoxic, mutagenic or carcinogenic, but the amines formed by anaerobic digestion may possess these characteristics (Harmer et al. 1992). A wide variety of faecal anaerobes have the ability to produce aromatic amines from azo dyes such as tartrazine, and may do so in the human gut, producing carcinogenic amines such as benzidine and 4-aminoaniline (Brown and DeVito 1993; Chung and Stevens 1992).

Aromatic amines can be mineralised by means of aerobic treatment (Easton 1995) by non-specific enzymes through hydroxylation and ring-opening of the aromatic compounds (Zissi and Lyberatos 1996). Field et al. (1995) showed that the aerobic stage of combined anaerobic-aerobic treatment of dye wastes eliminated the additional chemical oxygen demand (COD), attributed to the removal of aromatic amines, which are anaerobically recalcitrant. Components such as 6-aminonaphthalene-2-sulphonic acid have been shown to be totally degraded by a mixed aerobic culture (Rozgaj and Glancer-Soljan 1992). One study of the aerobic biode-

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gradability of a range of aromatic amines found that they were rapidly degraded (>90%) and were thus unlikely to remain in the environment for any length of time (Brown and Laboureur 1983). Therefore anaerobic treatment followed by aerobic treatment can be used to decompose putatively toxic and carcinogenic compounds efficiently.

The method for anaerobic azo-dye reduction has been described by Carliell et al. (1996). Carliell et al. (1994) proposed a theoretical range of anaerobic degradation products for Procion Red H-E7B that might be formed. It was confirmed by Carliell et al. (1995) that 2-aminonaphthalene-1,5,-disulphonic acid was present after anaerobic digestion of the dye, thus showing that the azo bond had been cleaved. Traces of 1,7-diamino-8-naphtho-3,6,-disulphonic acid and *p*-diamino-benzene were also found, although whether these were degradation products or contaminants was not determined. Cyanuric acid could not be identified by nuclear magnetic resonance (NMR) because of the absence of hydrogen atoms, but should have been present if the traces of the other compounds were due to degradation rather than contamination.

Because of their reported toxicity, it is important to demonstrate that aromatic amines generated by anaerobic decolorisation are degraded by aerobic treatment. However, although the detection of simple aromatic amines is relatively easy when additional groups (-SO_3, -OH, -COOH, -Cl) are present, quantitative detection is analytically much more complex. A simple method for detecting substituted aromatic amines is needed to demonstrate that azo-dye decolorisation in a biotreatment system is due to degradation, not simply adsorption, and to show that the amines generated are successfully removed by the process. The best-known method for the qualitative detection of aromatic amines is the diazonium coupling reaction (March 1968; Norwitz and Keliher 1986). However, because this method is a colorimetric assay giving a red colour, it is not suitable for use with most coloured samples.

In the work reported here, a simple HPLC-based procedure is proposed. This, coupled with total organic nitrogen (TON) measurements, could be used qualitatively to demonstrate the formation and degradation of amines from azo dyes. The procedure was applied to the treatment of a red-coloured reactive azo dye, Procion Red H-E7B (see Fig. 1 for structure), in a combined anaerobic-aerobic system.

Materials and methods

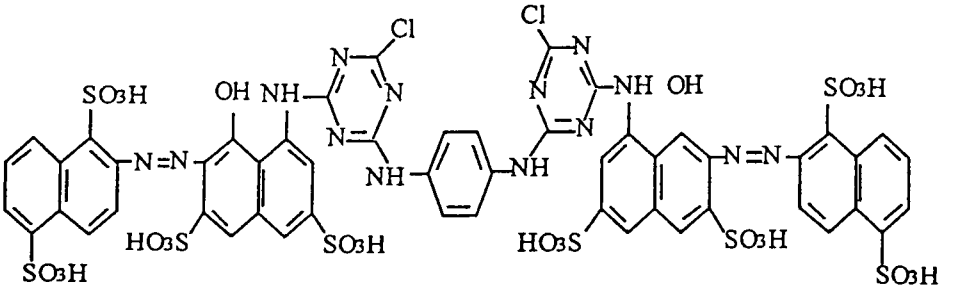
Reactor operation

The effluent from a 30-l UASB reactor [35 °C, 1-day hydraulic retention time (HRT)] was passed to a 20-l aerobic stage and a 3.75-l settler which operated at HRTs of 16 h and 3 h, respectively. The feed was a simulated textile effluent (STE) containing 0.45 g l^{-1} hydrolysed reactive azo dye (Procion Red H-E7B, BASF, UK) (Fig. 1), 2.9 g l^{-1} modified starch (Tissalys 150; Roquette, Kent, UK), 0.15 g l^{-1} NaCl, 530 mg l^{-1} acetic acid, trace elements and nutrients: NH_4Cl, 0.23 g l^{-1}; $(NH_4)_2SO_4$, 0.28 g l^{-1}; $Na_3PO_4 \cdot 12H_20$, 0.123 g l^{-1}; Na_2HPO_4, 0.038 g l^{-1}. The feed was stored (refrigerated) as a 15-fold concentrate, and diluted at the point of entry to the UASB with tap water, to which bicarbonate was added, giving a final concentration of 2 g l^{-1} $NaHCO_3$. To convert dye and starch into the forms normally found in industrial effluent, stock dye solution (50 g l^{-1}) was hydrolysed with NaOH to pH 12 at 80 °C for 1.5 h, and starch (100 g l^{-1}) hydrolysed with NaOH (40 g l^{-1}) at room temperature overnight. The dye, as supplied, contained 45% colour, 35% salt, 15% sodium lignosulphonate as a diluent and 5% moisture. Shading colors permitted for use with Procion Red H-E7B, as made by BASF, are Procion Yellow H-E4R and Procion Blue H-EGN, neither of which should be present at concentrations exceeding 2%.

The UASB was originally filled with 10 l of granules obtained from a paper-pulp processing plant and operated for 166 days, with a short break in operation at day 51, using a series of varying dye and starch concentrations in the STE. After a further break in operation (for 26 days) the UASB was fed STE from days 167 to 228. The aerobic stage was filled on day 171 with activated sludge from a local works operating on mixed domestic/trade effluent. A concentrated supplement of OECD simulated sewage [Organization for Economic Cooperation and Development (OECD), 1981] was fed to the activated sludge stage at a rate of 1.4 l per day to simulate a textile effluent treatment plant in which sewage contributes almost one-third of the COD to the aerobic stage. The simulated sewage, stored in a refrigerator for 7 days maximum, contained 4.8 g l^{-1} peptone, 3.3 g l^{-1} meat extract, 0.9 g l^{-1} urea, 0.21 g l^{-1} sodium chloride, 0.12 g l^{-1} calcium chloride dihydrate, 0.06 g l^{-1} magnesium sulphate heptahydrate, and 0.84 g l^{-1} potassium hydrogen phosphate. It was mixed with UASB effluent before entering the aerobic system.

Samples of STE, UASB effluent and settler effluent were sent for respiration-inhibition testing on day 209 (Alcontrol Laboratories, Yorkshire, UK). The respirometric inhibition tests were based on a previous method of the Standing Committee of Analysts (SCA). The SCA is due to publish an updated respirometry method based on the up-to-date protocol. The UKAS-accredited method used in this case involved an electrolytic respirometer (Meritox 20). Samples were taken on day 217 for amine analysis.

Fig. 1 Structure of Procion Red H-E7B



Assay methods

Parameters measured included true color, COD and biological oxygen density (BOD). The true color, and hence color removal, at each stage of treatment was determined by measuring the average optical density (OD) of centrifuged samples at 436, 525 and 620 nm in a manner similar to that described in the British Standards (BS 6068 1995). The COD of each sample was measured using a sealed-tube method as described in the Standard Methods (American Public Health Association 1989) with the mercury-free reagents described by Her Majesty's Stationery Office (1986). Each measurement was the mean of three replicates. The BOD_5 was measured using the WTW OxiTop 1230T system (Burmarc, UK) (total volume 250 cm^3; three replicates). Dilution water was made up as recommended by the Standing Committee of Analysts (1983) and contained one capsule of polyseed per 500 ml (Hach Company, Germany). Dilution water (50 ml) was added to each replicate and, after the addition of the sample, replicates were made up to volume using aerated deionised water. UASB effluent and settler effluent samples were filtered prior to this test. The initial total solids (TS) concentration, the total volatile solids (TVS) concentration of UASB granules the mixed liquor suspended solids (MLSS) in the aerobic stage as well as the TON content were measured as described in the Standard Methods (American Public Health Association 1989).

HPLC-UV analyses

HPLC analyses were carried out using a Varian 9012 gradient pump equipped with a C-18 chromatographic column and a Varian 2550 UV detector (Varian; Palo Alto, Calif., USA). Two different C-18 reversed-phase chromatographic columns (i.d. 3.0 mm, length 25 cm, stationary phase particle size 5 μm) were used: 250/3 Nucleosil 100-5 C18 AB (Macherey-Nagel, Easton, Pa., USA) and Supelcosil LC-18 (Supelco, Bellefonte, Pa., USA). The Supelco C18 column is the most widely used type of chromatographic column for non-specific HPLC detection of UV-absorbing compounds. The Nucleosil C18 column is suitable for the detection of aromatic amines formed during azo-dye degradation and is particularly appropriate for the specific detection of 16 aromatic amines commonly formed during this process.

When the Nucleosil column was used, the experimental conditions were those specified by the manufacturer for analysing aromatic amines [i.e., the eluents were acetonitrile (A), 5 mM pH 7 phosphate buffer solution (B); the gradient was A/B (5/95) in 35 min to A/B (85/15); the flow rate was 0.6 ml min^{-1}; and λ was 282 nm]. Different experimental conditions applied when the Supelco column was used [i.e., the eluents were acetonitrile (A), 5 mM pH 7 phosphate buffer solution (B); the gradient was A/B (70/30) in 40 min to A/B (30/70); the flow rate was 0.6 ml min^{-1}; and λ was 254 nm].

All the samples were pre-treated prior to HPLC analysis, in order to purify and concentrate them, as follows: 10 ml of sample was aspirated through a Chromabond HR/P cartridge (Macherey-Nagel) conditioned by consecutive feeding of 2 ml each of methanol, acetonitrile and 10^{-5} M NaOH. The cartridge was then washed with 2 ml of distilled water, dried under a vacuum and then eluted by 3 × 1 ml methanol:acetonitrile (1:1 v/v).

Results

The TS and TVS concentrations in the UASB were 33.7 g and 26.07 g, respectively per litre of reactor prior to the 166-day operating period, giving a specific organic loading rate of 0.13 kg COD kg^{-1} TVS per day and an organic loading rate of 3.34 kg COD m^{-3} per day. The mean MLSS of the aerobic stage between days 209 and 217 was 2.7 g l^{-1} (SD-0.4, *n*-4) with an average sludge age of 13.9 days. The mean COD and color of STE, UASB effluent and final effluent samples between days 209 and 217 can be seen in Table 1 along with the BOD taken on day 211.

The majority of the COD and BOD removal occurred anaerobically. The BOD:COD ratio for the STE entering treatment was 0.45:1, the ratio for the UASB effluent was 0.26:1, and the ratio for the final effluent was 0.13:1. Procion Red H-E7B is itself poorly biodegradable aerobically (BOD:COD = 0.08:1). It was clear that the majority of the color removal occurred in the anaerobic stage (63.9% as opposed to 11.1% in the aerobic stage). When the spectrum of the simulated textile effluent was examined in the visible range from 400–700 nm it was clear that the peak absorbance of the dye, which occurred between 500–560 nm, was greatly reduced after anaerobic treatment.

Respiration-inhibition tests

The STE and the final effluent were found to have undetectable toxicity in a 3-h respiration-inhibition test. The UASB effluent had a 3-h respiration inhibition of 17.9%.

Amine analysis

The 250/3 Nucleosil 100-5 C18 AB column was used and HPLC-UV analyses were carried out according to the analytical procedure provided by the manufacturer for the specific detection of 16 aromatic amines usually

Table 1 Mean chemical oxygen demand (*COD*), biological oxygen demand (*BOD*), and color of simulated textile effluent (*STE*), the ■ (*UASB*) effluent and final effluent samples. Values shown are the mean (with SD in parentheses). Each COD or BOD result is the mean of triplicate measurements

Parameter	STE	UASB effluent	Final effluent	Anaerobic reduction (%)	Aerobic reduction (%)	Overall reduction (%)
COD (mg l^{-1})	3343	906	507	72.9	11.9	84.8
	(313)	(155)	(66)	(6.9)	(4.2)	(3.6)
	4	4	4	4	4	4
BOD (mg l^{-1})	1517	240	68	84.2	11.3	95.5
	1	1	1	1	1	1
True color	2.88	1.04	0.72	63.9	11.1	75
	(0.6)	(0.02)	(0.12)	(7.0)	(7.5)	(3.8)
	5	5	5	5	5	5

originating during azo-dye degradation. Even though several peaks were present in all the recorded chromatograms, none of them corresponded to any of the 16 amines.

To avoid the time-consuming and analytically complex procedures necessary for the identification of each derivative (Carliell et al. 1994), simple HPLC-UV and TON analyses were carried out to determine qualitatively whether UV-absorbing amino-derivatives were formed and/or degraded during each treatment step. For this purpose, a "routine" C-18 Supelcosil HPLC-chromatographic column was used.

Figure 2 shows the HPLC chromatograms of STE (a), UASB effluent (b), aerobic feed (i.e., 30 vol. of UASB effluent + 1.4 vol. of OECD simulated sewage) (c), and final effluent (d), respectively. UV-absorbing substances eluting at between 1 and 2 min were examined. Chromatograms 2b and c demonstrate that the addition of synthetic sewage to the UASB effluent does not modify the HPLC chromatogram. Figure 2a and b shows that UV-absorbing by-products were formed during the anaerobic treatment of the STE since the total UV detectable area increased from a to b. Chromatograms 2c and d show that partial degradation of the UV-absorbing by-products formed during the UASB treatment occurred in the aerobic stage as the total UV detectable area decreased from c to d. Confirming the degradation of organic nitrogen-containing compounds, the TON decreased from 144 mg N l^{-1} before the aerobic stage to 108 mg N l^{-1} afterwards.

The chromatograms in Fig. 3 were recorded at a higher absorbance sensitivity (y-axis), and UV-absorbing substances eluting in the 2–5 min range were examined, providing additional information about the degradation by-products formed. The chromatogram for sample b is not shown because it was the same as c. In Fig. 3, as in Fig. 2, comparison of chromatograms a and c, shows that the UV-detectable area increased after anaerobic treatment, confirming the formation of additional aromatic by-products. Furthermore, the fact that the UV-detectable area in c shifted towards lower retention times means that these by-products of anaerobic treatment were more polar than the parent compounds shown in (a). Figure 3c and d also shows that when such by-products were degraded aerobically they formed less-aromatic, more-polar compounds, since the UV-detectable area decreased in 3d and was shifted towards lower retention times.

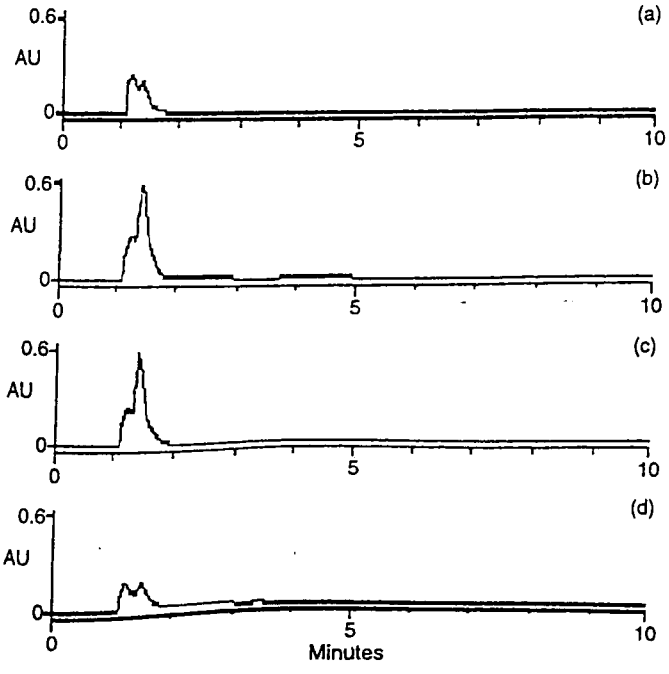


Fig. 2a–d HPLC-UV chromatograms obtained using a C-18 Supelcosil column. See the text for detailed explanations of parts a–d

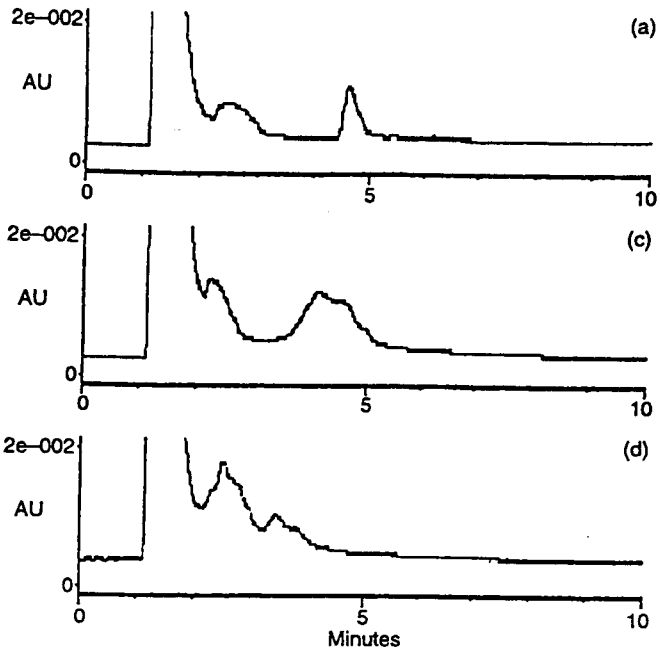


Fig. 3a–d HPLC-UV analyses obtained using a C-18 Supelcosil column and a high UV-absorbance sensitivity. Parts a–d are as in Fig. 2

Discussion

Anaerobic biodegradation of dyes is cited in the literature as generating aromatic amines when color removal occurs by cleavage of the azo bond. The fact that most of the color was removed anaerobically confirms the observations of Loyd et al. (1992).The primary reason for using an aerobic stage was to remove these amines. Respiration-inhibition testing showed that anaerobic degradation of the STE generated metabolites that were toxic to at least some of the aerobic organisms used in the test. The dye is the only substance present in the STE that could produce toxic by-products by means of anaerobic digestion and therefore it was concluded that the

dye had been degraded. The toxicity was eliminated after aerobic treatment, indicating that the products causing toxicity in the respiration-inhibition test were degraded aerobically. This conforms with known information on anaerobic-aerobic degradation of dyes, and suggests that the dye was not simply adsorbed to biomass but was actually degraded anaerobically to amines that were then removed by means of activated sludge treatment. It is probable, therefore, that some of the COD removal in the aerobic stage was attributable to degradation of these metabolites, which appears to concur with the observations reported by Field et al. (1995).

The findings obtained with the "250/3 Nucleosil 100-5 C18 AB" column are explained by the hypothesis that during biological degradation of the dye several derivatives containing amino groups ($-NH_2$) were formed. This was indicated by the occurrence of unidentified peaks, agreeing with the results of Carliell et al. (1994), which demonstrated that during anaerobic degradation of Procion Red H-E7B, several amino-derivatives bearing additional groups such as -OH and $-SO_3$ were formed.

The chromatograms demonstrate that anaerobic treatment generated UV-absorbing compounds that were more polar than the parent compounds. The anaerobic degradation of reactive azo dye has been shown to produce several aromatic and ionic by-products (Carliell et al. 1994). Since amino-derivatives are reported to cause respiration-inhibition effects (Walker 1989), the respiration inhibition of aerobic bacteria caused by UASB effluent supports this observation. The chromatograms also demonstrate that aerobic treatment reduced the levels of these UV-absorbing compounds and increased their polarity. Degradation in the aerobic stage may result in the formation of oxidised and very polar derivatives (e.g., aldehydes, carboxylic acids) having a lower aromaticity, as suggested by Nörtemann et al. (1986) in a study of 6-aminonaphthalene-2-sulphonic acid degradation. Together with the aerobic decrease in TON, it can be reasonably concluded that, during the aerobic stage, some degradation of nitrogen-containing aromatic derivatives (likely to be aromatic compounds bearing amino-groups) took place with mineralisation of organic nitrogen.

It can be concluded that the results from simple HPLC-UV analyses coupled with TON measurements, colour removal, COD removal and respiration-inhibition tests can be used to demonstrate qualitatively that aromatic amino-derivatives are formed during anaerobic treatment and are degraded into more polar, non-aromatic by-products during the succeeding aerobic stage.

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Anaerobic and aerobic treatment of a simulated textile effluent

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Abstract: A simulated textile effluent (STE) was generated for use in laboratory biotreatment studies; this effluent contained one reactive azo dye, PROCION Red H-E7B (1.5 g dm^{-3}); sizing agent, Tissalys 150 (1.9 g dm^{-3}); sodium chloride (1.5 g dm^{-3}) and acetic acid (0.53 g dm^{-3}) together with nutrients and trace elements, giving a mean COD of 3480 mg dm^{-3}. An inclined tubular anaerobic digester (ITD) was operated for 9 months on the STE and a UASB reactor for 3 months. For a 57 day period anaerobic effluent from two reactors, a UASB and an ITD, was mixed and treated in an aerobic stage. In days 77–247 68% of the true colour of PROCION Red H-E7B was removed by anaerobic treatment with no colour removal aerobically and up to 37% COD was removed anaerobically, with a corresponding BOD removal of 71%. For combined anaerobic and aerobic treatment a mean COD removal of 57% and BOD removal of 86% was achieved. Operation of the ITD at a 2.8 day HRT (volumetric loading rate (B_v) 1.24 g COD dm^{-3}day^{-1}) and the UASB at a 2 day HRT (B_v 1.74 g COD dm^{-3}day^{-1}) gave comparable COD removals but the UASB gave better true colour removal. Effluent from the combined process operating on this simulated waste still contained an average 1500 mg COD dm^{-3}, and further treatment would be required to meet consent standards.

Keywords: reactive azo dyes; anaerobic digestion; aerobic treatment; textile; wastewater

NOTATION

ADMI	American Dye Manufacturers' Institute
BA	Bicarbonate Alkalinity
BOD	Biological Oxygen Demand
B_v	Volumetric loading rate
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
HRT	Hydraulic Retention Time
ITD	Inclined Tubular Digester
MLSS	Mixed Liquor Suspended Solids
STE	Simulated Textile Effluent
TOC	Total Organic Carbon
TOD	Total Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
TVFA	Total Volatile Fatty Acids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids

1 INTRODUCTION

Textile effluent varies from day to day and even hour to hour due to the batchwise nature of the dyeing process and is therefore difficult to characterise. The composition is determined by the processes involved, fibre type and chemicals used. The most pronounced variations include the colour of the wastewater and the type of dye contained in it. The strong colour of textile wastes is the hardest component to treat.[1] The effluent typically contains a large number of compounds as demonstrated by one report which, on analysis of wastewater streams from four factories, positively identified 314 compounds, determined the partial structure of 94, and detected an additional 107 unknown compounds.[2] The principal pollutants in textile effluent are aromatics, halogenated hydrocarbons and metals.[3]

Use of a simulated waste facilitates the assessment of the treatment process without interference from the major fluctuations in effluent composition associated with real industrial wastes. Generally textile effluent composition and dye concentrations in the effluent are so variable that no artificial waste can be truly representative, either of a particular type of waste or even of a particular factory. This makes it difficult to select a simulated textile effluent (STE) composition for experimental processes. However, a number of researchers have used artificial textile effluents in the

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investigation of treatment technologies. Many simulated wastes contain no compounds found in textile effluents other than dyes although some contain a carbon source and nutrients.

Cotton is the principal fibre type used throughout the world and in 1995 was estimated to account for 44.9% of world fibre production.[4] It is associated with the use of reactive dyes and consequently has become identified with coloured effluent since up to 50% of these dyes, in the hydrolysed form, may be present in the effluent. Due to the poor colour removal achieved in aerobic treatment of cotton effluent, it was decided to imitate this type of wastewater. A reactive azo dye was selected for use in the STE studied here. It was decided to imitate a mixed waste rather than a dyebath effluent in order that the waste would contain size as a carbon source, as labile carbon is required to maintain the rate of azo reduction in anaerobic systems.[5] Sizes, or sizing agents, are substances used to protect yarn from the stresses of weaving and starch is commonly used as a size for cotton fabric.

The STE used in these experiments was treated using anaerobic and aerobic systems. An inclined tubular anaerobic digester (ITD) was used in order to retain biomass without using an additional settler.[6,7] The inclined tubular digester (ITD) minimises the gas/liquid surface area where crusts and scum can form, no mixing is required and the inclination of the digester increases the solids retention time by means of sedimentation.[6] The gas yields per unit of VS added usually exceed those from comparable CSTRs due to the increased solids retention. The ITD has been used in a number of investigators.[6–9] The STE was also treated in a conventional UASB reactor, and the effluent from these reactors further treated in an activated sludge stage. UASBs have been used by other authors in treatment of dyes and textile wastes.[10–13] The degree of colour and COD removal by this treatment and the loading rates achieved are reported here.

2 MATERIALS AND METHODS

2.1 Effluent composition

The principal components of the simulated textile effluent were size ($1.9\,g\,dm^{-3}$), dye ($1.5\,g\,dm^{-3}$), NaCl ($1.6\,g\,dm^{-3}$) and acetic acid ($0.53\,g\,dm^{-3}$). Nutrients (N $120\,mg\,dm^{-3}$ as NH_4Cl and $(NH_4)_2SO_4$; P $18\,mg\,dm^{-3}$ as $Na_3PO_4.12H_2O$ and Na_2HPO_4) and trace elements were also added using technical grade chemicals. The STE was refrigerated (~6 °C). Due to an experimental error acetic acid was not added to the STE between days 20 and 30. The size was Tissalys 150, a modified potato starch widely used in the cotton industry (Allied Colloids, Bradford, UK, pers comm). The dye was PROCION Red H-E7B (BASF, Manchester, UK), a reactive azo dye, the structure of which has been published.[14]

The dye as supplied contains 45% colour, 35% salt, 15% sodium lignosulfonate as a diluent, and 5% moisture (w/w). Small quantities (not exceeding 2% w/w) of other colours may be added to obtain the correct shade each time a batch is made up. The dye was hydrolysed to convert it to the form in which it is normally found in industrial effluent by adjusting a $50\,g\,dm^{-3}$ stock solution of PROCION Red H-E7B to pH 12 with $1\,mol\,dm^{-3}$ NaOH and heating the solution at 80 °C for 1.5 h. The size was hydrolysed for similar reasons by mixing a stock solution of $100\,g\,dm^{-3}$ size and $40\,g\,dm^{-3}$ NaOH and leaving it at room temperature overnight.

2.2 Reactor operation

The ITD and UASB had 8.5 and $5\,dm^3$ working volumes respectively. The ITD was seeded with sludge from a municipal wastewater treatment plant which treated textile effluent and may have already contained an acclimated population. The UASB was seeded with granules from a paper pulp processing plant unadapted to dye treatment. Both digesters were heated with Grant FH15 flow heaters, the ITD externally and the UASB by means of a water jacket, to give an internal temperature of 35 °C. The ITD was inclined at 20° to obtain good temperature distribution.[7] Ports were located at 200 mm intervals to facilitate sample removal. Weirs at the head of the ITD retained solids, eliminated the requirement for a subsequent settling tank and sludge recycle and increased the gas pressure at the head of the reactor. The UASB had an arrangement of two funnels, one inside the other, as a three-phase separator. Gas from both digesters was forced under pressure to a continuously recording low flow gas meter.[15] On-line temperature and pH were logged to a PC using LabVIEW. The ITD was fed intermittently for 37 days followed by 7 days of continuous feeding prior to commencing measurements on day 1. It was operated continuously from days 1 to 77 at a 3.5 day, 2.8 day and 1.4 day HRT. After day 77 more anaerobic sludge from a plant treating municipal sewage sludge was added. The ITD was then operated from days 78 to 247 at a 2.8 day HRT. The UASB was operated continuously from day 150 at a 1 day HRT, from day 201 at a 2 day HRT and from day 235 at a 1.73 day HRT.

The $10\,dm^3$ aerobic vessel, aerated by three Capex L2C air compressors (Charles Austen Pumps, Fisher, Leics) attached to air stones, was filled on day 193 with activated sludge from the sludge return of a local wastewater treatment plant. Effluent from both anaerobic reactors was mixed and pumped to this vessel. Aerobic effluent was pumped to a settling tank fitted with a 1 rpm stirrer to assist settling. Sludge was returned to the aerobic stage at 8-hourly intervals by means of a timer (RS, Northants, UK) linked to a pump.

2.3 Analytical methods

2.3.1 *Organic content*

The five day biochemical oxygen demand (BOD_5) (≤20% error)[16] was measured using the WTW

Analysis	*Value*	*sd*	*n*	*Reported range*[24]
BA mg dm^{-3} $CaCO_3$	1410	305	95	
COD	3480	671	112	500–5000
BOD	1127	288	2	200–2000
COD:BOD	3.1: 1			
TSS	260	40	1	50–500
pH	9.4	0.7	87	4–12
TVFA	573	173	111	
TOC (day 201)	2099	34	3	
TOD (day 208)	3450		1	
Apparent colour[a]	5.65	0.16	2	
True colour[a]	5.62	0.12	2	
ADMI (×1000)[b]	131.2			
ADMI (×1000)[c]	126.1			

[a] measured on days 222 and 229.
[b] pH 9.4.
[c] pH 7.6.
Mean COD measurement is calculated after day 31.
sd, standard deviation.
n, population number.

Table 1. Characterisation of STE and reported range of some parameters (mg dm^{-3} unless otherwise stated)

OxiTop 1230T system (Burmarc Ltd, UK) (sample volume 250 cm^3). Dilution water was made up as recommended.[17] The chemical oxygen demand (COD) (≤9.01% error)[18] of samples was measured using a sealed tube method[19] with mercury-free reagents.[18] The TOC was measured on a DC-190 Total Organic Carbon analyser (Rosemount Dohrman) and the TOD on an Ionics Model 7800 E Total Oxygen Demand Analyser (Ionics, UK). All measurements were made in triplicate.

2.3.2 Colour

The spectra of samples were examined using an ATI Unicam UV2-100 UV/Visible spectrometer v 3.10 with a 1 cm plastic cell (0.5% error at an absorbance of 1). The apparent colour of samples was determined by measuring the average absorbance at 436, 525 and 620 nm and true colour by repeating the measurement in centrifuged samples. These measurements were carried out at the normal pH of the STE. The percentage colour removal was calculated from true colour measurements. The ADMI (American Dye Manufacturers' Institute) units of STE and anaerobic effluent were determined according to established methods[19,20] at the normal sample pH and at pH 7.6.

2.3.3 Other measurements

The total suspended solids concentration (TSS) (0.76–33% error) and total volatile suspended solids concentration (TVSS) (7.2% error) were measured using a standard method.[19] Volatile fatty acids (<2% error) and biogas composition (<0.5% error) were measured as described elsewhere.[21] On-line pH measurements were taken using a Solomat instrument (Neotronics, UK). The bicarbonate alkalinity of the reactor was measured by titration with standardised 0.05 mol dm^{-3} HCl to pH 5.75.[22] Anaerobic biodegradability tests were carried out in accordance with a recommended method.[23]

3 RESULTS AND DISCUSSION

3.1 Simulated textile effluent

The STE contained approximately 1.72 g dm^{-3} sodium plus a small indeterminable quantity from the diluent in the dye powder. The concentrations were therefore below the 3.5–5.5 g dm^{-3} cited as causing moderate inhibition of methanogenesis.[5] Results obtained from analysis of the STE over the experimental period are shown in Table 1. It was seen that the COD, BOD, TSS and pH were all within reported ranges for textile wastewaters. The TOC was lower than the COD as it does not take into account the presence of nitrogenous compounds which could be oxidised. The TOD was comparable to the COD readings, suggesting that it could be used as a surrogate for off-line COD tests in this instance. The TVFA measured in the STE was due to the addition of acetic acid to the STE. The pH-adjusted sample had a lower ADMI showing that pH is an important factor to consider when determining colour.

The dye was poorly biodegradable as was confirmed by the lack of biogas production in anaerobic degradability tests. This dye has low toxicity and is unlikely to cause inhibition to aerobic bacteria.[25] Anaerobic biodegradability tests showed the starch to be completely biodegradable as would be expected. Acetic acid is also readily biodegradable. The STE without dye was found to be fully degradable while the STE without starch was not degraded, indicating that a carbon source is essential for anaerobic degradation of the dye. The STE itself was fully degraded although the net volume of gas produced was lower than that produced by the STE without dye showing that the dye may limit degradation slightly.

3.2 Reactor operation

3.2.1 Anaerobic operation at varying loading rates

The ITD was seeded with a biomass concentration of

Analysis	Units	days	Value	sd	n
BA	mg $CaCO_3 dm^{-3}$	1–28 (3.5 days HRT)	1362	193	16
		40–69 (2.8 days HRT)	1167	162	18
pH		1–28 (3.5 days HRT)	7.38	0.2	16
		40–69 (2.8 days HRT)	6.96	0.2	18
Effluent COD	$mg dm^{-3}$	1–28 (3.5 days HRT)	3300	1058	11
		40–69 (2.8 days HRT)	2026	709	17
COD reduction	%	1–28 (3.5 days HRT)	46	20.8	11
		40–69 (2.8 days HRT)	38	15.3	17
Effluent BOD	$mg dm^{-3}$	70	422	29	3
COD:BOD		70	4.8:1		1
TVFA	$mg dm^{-3}$	1–28 (3.5 days HRT)	516	151	16
		40–69 (2.8 days HRT)	607	137	20

Table 2. ITD Digester performance from days 1 to 69

sd = standard deviation, n = no. of samples, except in the case of COD, TOD and TOC where each n is the average of three replicates.

$11.3 g dm^{-3}$ TSS ($8 g dm^{-3}$ TVSS). After continuous operation for 77 days an increase of 48% and 27.5% respectively was found. The ITD was supplemented with sludge on day 78 to give a biomass concentration of $20.4 g dm^{-3}$ and $13 g dm^{-3}$ of TSS and VSS respectively. The sludge did not settle very well in the first 7 days of operation, however, with the result that some solids were lost. When the reactor was emptied at the end of the experiment a 14% loss in both TSS and VSS was found.

3.2.2 *ITD operation*

The ITD was operated at an organic loading rate (B_v) of 0.99 g COD dm^{-3} day^{-1} (HRT 3.5 days) from days 1 to 28. At this B_v the effluent was a pale brown colour. The% COD removal and TVFA can be seen in Table 2. The B_v was increased to 1.24 g COD dm^{-3} day^{-1} (HRT 2.8 days) by increasing the flow rate on day 28. During days 20–30 when acetic acid was not added to the STE some acidification occurred naturally to give a total VFA concentration of $92 mg dm^{-3}$. The concentration of acetic acid in the ITD was not greatly affected by the absence of acetate in the STE. The ITD was given a step loading on day 70 by doubling the B_v to 2.49 g COD dm^{-3} day^{-1} (HRT 1.4 days). At this loading rate the effluent was more deeply coloured than previously and the TVFA rose to approximately $900 mg dm^{-3}$. From day 78 to 247 the ITD was operated at a B_v of 1.24 g COD dm^{-3} day^{-1} (HRT 2.8 days). This gave a sludge loading rate of 0.067 g COD g^{-1} TSS day^{-1} calculated on the sludge present at the end of the experiment. Table 3 shows the ITD performance from days 77 to 247. The ITD appeared adapted to operation at a 2.8 day HRT within 2 months of start-up as effluent quality and percentage COD reduction in days 40–69 and 88–247 (Table 3) are similar. The pH in days 88–247 was higher, probably due to the lower concentration of TVFAs. This indicates that bacteria can become adapted to certain loading conditions.

3.2.3 *UASB operation*

The $5 dm^3$ UASB was filled with $2.5 dm^3$ of granules giving a TS of $81.5 g dm^{-3}$ and a VS of $67.9 g dm^{-3}$. When the reactor was emptied at the end of the experimental period a decline of 37% and 42% respectively was found. This was due to washout of granules from the reactor, attributable to incomplete separation at the gas–liquid separator. The UASB was originally fed at an HRT of 1 day (B_v 3.48 g COD dm^{-3} day^{-1}) but due to a rise in the concentration of VFAs after day 180 from approximately $200 mg dm^{-3}$ to over $1000 mg dm^{-3}$ the HRT was increased to 2 days (B_v 1.74 g COD dm^{-3} day^{-1}) on day 201. By day 237 the VFA concentration had dropped to below $200 mg dm^{-3}$ so the HRT was decreased to 1.73 days (B_v 2.01 g COD dm^{-3} day^{-1}). The VFAs did not increase between the time when the

Table 3. Anaerobic effluent and aerobic effluent parameters in the period days 77–247

Analysis	ITD 2.8 day HRT	UASB 1 day HRT	UASB 2 day HRT	Aerobic
BA ($mg dm^{-3}$ $CaCO_3$)	**1578** (138) 42	**2033** (222) 9	**1213** 1	
Effluent COD ($mg dm^{-3}$)	**2174** (450) 69	**2580** (644) 18	**2252** (450) 7	**1506** (504) 2
% COD reduction	**37** (11.3) 69	**30** (17.3) 17	**32** (11.8) 7	**23** (10.1) 22
Effluent BOD ($mg dm^{-3}$)[a]	**324** (86) 2		**324** (86) 2	**143** (101) 2
% BOD reduction (days 137, 237)	**71** (0.03) 2	NA	**71** (0.03) 2	**14** (13) 2
COD:BOD	**6.7: 1**		**6.7: 1**	**10.5:1**
Effluent TVFA ($mg dm^{-3}$)	**353** (233) 71	**278** (357) 17	**506** (369) 7	

BA bicarbonate alkalinity

[a] Numbers are: **value** (sd) number of samples BOD was calculated from mixed effluent samples rather than from the individual reactors, therefore values for ITD and UASB are presented as identical.

Analysis	*ITD 2.8 day HRT*	*UASB 2 day HRT*	*Aerobic*
Apparent colour (days 222, 229)	**2.07** (0.2) 2	**1.19** (0.04) 2	**1.74** (0.25) 2
% Reduction (days 222, 229)	**63.3** (4.6) 2	**79** (0.06) 2	**–7**
True colour (days 222, 229)	**2.11** (0.16) 2	**1.23** (0.06) 2	**1.78** (0.25) 2
% Reduction (days 222, 229)	**62.4** (3.6) 2	**78** (0.7) 2	**–6.6**
ADMI[a] (×1000), days 108, 123	**6.62** (1.6) 2		
ADMI[b] (×1000), days 108, 123	**7.13** (1.01) 2		
% reduction in pH adjusted sample	**94.3**		

Table 4. Apparent colour, true colour and % true colour removal of simulated effluent, anaerobic effluent and aerobic effluent within the period days 78–247

Numbers are: **value** (sd) number of samples.
[a] Normal pH of effluent (7.9–8.9).
[b] Adjusted to pH 7.6.

HRT was shortened and the end of the series of experiments. The sludge loading rates were 0.067, 0.034 and 0.039 g COD g^{-1} TS day^{-1} respectively, calculated on the sludge present at the end of the period. It can be seen that similar sludge loading rates were achieved in the UASB at a 1 day HRT and the ITD at the longer HRT of 2.8 days. At HRTs of 1.73 days and 2 days the UASB sludge loading rates were lower than those achieved in the ITD although the HRT was shorter. At a 1 day HRT in the UASB the loading rates of the two reactors were comparable. Given the high loss of biomass from the UASB it is difficult to compare the two digesters in terms of sludge loading rates. However, the two digesters were compared in terms of VFAs produced and removal of organics and colour in relation to the volumetric loading rates in order to determine which was the most appropriate digester for treatment of this type of waste. The ITD appears more suited to retaining biomass during operation on STE than the design of the UASB reactor used here at laboratory scale.

3.2.4 Aerobic reactor

The aerobic stage was fed from day 193 with effluent from both anaerobic reactors. The HRTs of the activated sludge stage were 1.24, 1.8 and 1.69 days when the UASB HRT was 1, 2 and 1.73 days respectively. The majority of results were obtained at an aerobic HRT of 1.8 days. At this HRT the effluent contributions from the ITD (2.8 day HRT) and the UASB (2 day HRT) were 3.04 and 2.5 dm^{-3} day^{-1} respectively. The aerobic reactor was originally seeded with activated sludge containing 2.41 g dm^{-3} of MLSS, and 2.26 g dm^{-3} TVSS. This declined over the operating period to 1.32 and 0.94 g dm^{-3} respectively. Therefore no settled solids were disposed of to waste although unsettled solids were lost from the settling tank. When this was measured as a percentage of the solids contained in the aerobic stage, an average of 28% was obtained, giving a sludge age of under 4 days. As the biomass in the aerobic tank declined over the operating period, the growth of biomass was less than the rate of loss of solids in the settling tank effluent.

The pH within the activated sludge tank was between 8 and 9. This was due to the high pH of the STE (Table 1) attributable to the large quantity of NaOH used to hydrolyse the starch. In the anaerobic digesters the pH did not decline below 6.8, and was normally about 7.1. However under aerobic conditions the VFAs volatalised or were metabolised resulting in a rise in pH. Therefore pH control at the activated sludge stage would be required to provide conditions more suitable for the growth and settling of aerobic consortia.

3.2.5 Organics removal

The COD: BOD ratios increased after anaerobic and aerobic treatment (Tables 2 and 3) showing that biodegradable material was removed. Anaerobic treatment in the ITD removed more biodegradable material during days 77–247 than in days 40–69 as evidenced by the higher ratio. The highest percentage of anaerobic COD reduction was achieved in the ITD (Tables 2 and 3). The percentage COD reduction in the UASB increased only slightly when the HRT was doubled. The higher removal by the ITD is possibly attributable to the fact that it had the longer HRT (Table 3). An overall 57% (standard deviation 17.9; 22 samples) reduction in COD and 86% (standard deviation 13; 2 samples) removal of BOD was achieved as a result of anaerobic and aerobic treatment. The theoretical readily removable COD was 74% based on the contributions of starch and acetic acid to the STE. Therefore it appears that the anaerobic biodegradation rate was low despite the STE being fully biodegradable. Acidification seems to be the rate-limiting step.[26] Combined anaerobic and aerobic treatment do not remove all the easily biodegradable material. This indicates that the dye or its degradation products may be limiting degradation.

3.2.6 Colour removal

Anaerobic microbial decolourisation of reactive azo dyes occurs due to reduction and cleavage of the azo bonds with which the colour is associated, and results in the production of amines and amides which are biodegradable in aerobic conditions.[5] The peaks in the region between 515 and 545 nm corresponding to the presence of dye were removed by anaerobic treatment at a 2.8–3.5 day HRT and replaced by a small peak around 440 nm. However following the step loading to

the ITD of 2.49 g COD dm^{-3} day^{-1} the 515–545 nm peaks were not removed although their intensity decreased. Thus an ITD HRT of 1.4 days was not sufficient to decolourise the dye.

The results obtained from measurement of the apparent and true colour of the samples within the period days 78–247 can be seen in Table 4. Colour measurements for the STE are given in Table 1. A higher colour removal was achieved in the UASB than in the ITD although it was unclear how much removal was attributable to adsorption in each case. The ADMI reading is obtained over a greater range of wavelengths which makes it the more accurate measurement than apparent or true colour. However, due to the range of wavelengths measured and the complex equations involved, the ADMI measurement is time-consuming to perform on a regular basis. True colour measurements provide a more rapid assessment of reactor performance in terms of colour removal. The aerobic effluent had a higher colour than the mixed anaerobic effluent. This could be due to the increase in pH in the aerobic stage that occurs when the VFAs evaporate or are metabolised, or due to the oxidation or polymerisation of compounds formed in the anaerobic stage. It has been suggested that the formation of a more highly coloured product after treatment of a substance containing a diazo chromophore is due to either spontaneous or microbially catalysed oxidation of the unstable aromatic amines.[27] The effect of pH on ADMI measurements was noted (Tables 1 and 4).

It was seen from the true colour measurements (Table 4) that colour removal occurred in the anaerobic treatment stage, presumably involving reduction of azo bonds to amino groups[5,28] The greater colour removal by the UASB made it a more effective reactor for treatment of this type of wastewater providing the problem of granule loss could be overcome, eg by better three-phase separator design. These results show that anaerobic treatment is effective in the removal of colour from STE while aerobic treatment has little further effect in terms of colour removal. In spite of this, the role of aerobic treatment in amine breakdown and in removing COD and solids means it can be very useful in textile effluent treatment subsequent to anaerobic treatment.

The effluent from the combined treatment was still coloured and therefore effluent from the treatments carried out here would need to be highly diluted or to be treated further prior to discharge. It has been noted that rates of decolourisation are inversely proportional to the initial dye concentrations.[5] Therefore decreasing the concentration of the dye in the STE might result in a faster rate of colour removal. Given that the concentration of dye used here was higher than that used in many other simulated textile effluents, this is a practical proposition in terms of imitating real wastes. It is difficult to find any figures relating to actual concentrations of dyes in real wastes as most authors discuss colour in terms of absorbance or ADMI values. However, one author has given 0.1–0.2 g dm^{-3} as the normal range of reactive dyebath residues.[29] The ADMI of the pH-adjusted STE here was higher than the 1390 units for reactive dye wastewater from a continuous process and 3890 for reactive dye wastewater from a batch process cited elsewhere.[30] When the typical absorbances of site effluent[31] and STE in the range of 400–700 nm were examined it was found that the mean value for the STE was higher than that of the site effluent. At 400–550 nm the absorbance of the STE exceeded that of typical site effluent, and from 600–700 nm was lower than the typical values. The differences are partly attributable to the use of only one dye, but also indicated that the concentration of the dye used in this case is in excess of typical industrial concentrations. Therefore this study comprises a 'worst case' scenario.

4 CONCLUSIONS

(1) Up to 78% of the true colour measurement of PROCION Red H-E7B was removed by anaerobic treatment. No colour removal occurred aerobically.

(2) A mean COD removal of 35% and a BOD removal of 71% was achieved by anaerobic treatment alone with a mean COD removal of 57% and BOD removal of 86% being achieved after the combined treatment.

(3) Operation of the ITD at a 2.8 day HRT (B_v–1.24 g COD dm^{-3} day^{-1}) and the UASB at a 2 day HRT (B_v–1.74 g COD dm^{-3} day^{-1}) gave better COD removal in the ITD but the UASB gave better true colour removal.

(4) Due to the high loss of solids from the UASB the two anaerobic digesters could not be compared in terms of sludge loading rates.

(5) Effluent from the combined process operating on this simulated waste still contained an average 1500 mg COD dm^{-3}, and further treatment would be required to meet consent standards.

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ANAEROBIC–AEROBIC BIOTREATMENT OF SIMULATED TEXTILE EFFLUENT CONTAINING VARIED RATIOS OF STARCH AND AZO DYE

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Abstract—Combined anaerobic–aerobic treatment was used to treat a simulated textile industry wastewater (overall HRT 1.8 days). The azo dye (PROCION Red H-E7B) and starch concentrations were varied in a series of 1-week experiments to determine the effect of starch:dye ratio on COD, BOD and colour removal. The treatment efficiency of the system at 1.9 g l^{-1} starch and 0.15 g l^{-1} dye remained constant over 130 days despite seven intervening 1-week periods of operation at other starch:dye ratios. Most colour removal occurred in the UASB reactor (1 d HRT), and the BOD:COD of the UASB reactor effluent increased by up to 47%. The maximum overall COD removal was 88% and the BOD removal was up to 99%. A maximum of 77% colour removal overall was achieved with starch and dye concentrations of 3.8 and 0.15 g l^{-1}, giving a final true colour of 0.21 TCU. At both 0.15 and 0.75 g l^{-1} dye a starch concentration of 3.8 g l^{-1} rather than 1.9 g l^{-1} gave a significant improvement in colour removal. However, at 3.8 g l^{-1} starch volatile fatty acid levels in the UASB reactor rose, while at 2.9 g l^{-1} starch they did not. It is recommended that if colour removal efficiency decreases, carbohydrate should be added to the anaerobic reactor at a maximum sludge loading rate between 0.11 and 0.15 kg COD kg^{-1} TVS d^{-1}. © 1999 Elsevier Science Ltd. All rights reserved

Key words—azo dye, anaerobic-aerobic biotreatment, UASB reactor

INTRODUCTION

The textile industry produces a multi-component waste, which can be difficult to treat. The dyes contained in the effluent can vary daily or even hourly. Azo dyes are the class of dyes most widely used industrially (FitzGerald and Bishop, 1995) having a world market share of 60–70% (Geisberger, 1997; ETAD, 1998).

Anaerobic and aerobic treatment have been used together or separately for treatment of textile effluents. An average of 10% and a maximum of 30% of reactive dyes (e.g. azo dyes) are adsorbed onto aerobic biomass (Pierce, 1994; Waters, 1995), the remainder passing through activated sludge plants. Hence aerobic treatment is not effective in colour removal from textile wastes containing azo dyes.

Anaerobic treatment alone has been shown to remove COD from textile effluents (Delée *et al.*, 1998) and has the advantage of lower sludge production and lower energy demand compared to aerobic treatment. A wide range of azo dyes is decolourised anaerobically, being reduced by co-metabolism or when acting as electron acceptors for anaerobic respiration by non-specific enzymes, forming aromatic amines. A labile carbon source is therefore essential (Carliell *et al.*, 1996). The amines formed may be recalcitrant anaerobically but can be degraded aerobically. Anaerobic treatment can provide 97% decolourisation and 60% COD removal, and subsequent aerobic treatment can remove an additional 30% COD, thought to be due to the removal of aromatic amines (Field *et al.*, 1995; Delée *et al.*, 1998).

Upflow Anaerobic Sludge Blanket (UASB) reactors are the most commonly used high-rate anaerobic system. They are generally used for wastewaters that have a low suspended solids concentration (Angenent and Dague, 1995) and can be used for treatment of dye wastes (An *et al.*, 1996; Razo-Flores *et al.*, 1997; Zhu *et al.*, 1994). The experiments carried out here used a UASB reactor and aerobic reactor with a fully defined influent, simulating cotton dyeing wastewater, in which the ratio of starch to dye was varied. The effect of the ratio of electron donor (starch) and electron acceptor (dye) on colour removal was examined.

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Table 1. Dye and starch concentrations and volumetric loading rate (B_v) to UASB reactor for all experiments

Experiment	Days	Dye (gl^{-1})	Starch (gl^{-1})	B_v (g COD l^{-1} reactor d^{-1})
Initial	1–22	Low (0.15)	Low (1.9)	2.29
1	29–36; 44–51	Low (0.15)	Low (1.9)	2.22
	52–59; 66–73			
	80–87; 95–101			
	108–115			
2	36–44; 73–80	High (0.75)	Low (1.9)	2.71
3	59–66; 87–95	Low (0.15)	High (3.8)	3.73
4	101–108; 115–123	High (0.75)	High (3.8)	3.90
5	171–186	Medium (0.45)	Medium (2.9)	3.14

MATERIALS AND METHODS

Apparatus

The effluent from a 30-l UASB reactor (35°C, 1 day HRT) passed to a 20-l aerobic stage and 3.75-l settler which operated at HRTs of 16 h and 3 h, respectively. The feed, a simulated textile effluent (STE) contained hydrolysed reactive azo dye (PROCION Red H-E7B), modified starch (Tissalys 150, Roquette, Kent, UK), 0.15 gl^{-1} NaCl, 0.53 gl^{-1} acetic acid, trace elements and nutrients, ($(NH_4)_2SO_4$, 0.28 gl^{-1}; NH_4Cl, 0.23 gl^{-1}; $Na_3PO_4.12H_20$, 0.123 gl^{-1}; Na_2HPO_4, 0.038 gl^{-1}). The feed, stored refrigerated as a 10-fold concentrate, was diluted at the point of entry to the UASB reactor with tap water containing bicarbonate giving a final concentration of 2 gl^{-1} $NaHCO_3$. A stock dye solution (50 gl^{-1}) with NaOH to pH 12 was hydrolysed at 80°C for 1.5 h, and the size (100 gl^{-1}) hydrolysed with NaOH (40 gl^{-1}) at room temperature overnight.

The UASB reactor was seeded with 10 l of granules from a paper pulp processing plant, operated for 4 months on STE (starch 0.95–1.9 gl^{-1} and dye 0.075–0.15 gl^{-1}), then switched off for 52 days prior to this set of experiments. The aerobic sludge was collected on day 8 from a wastewater treatment plant, which treats trade and domestic effluents. Solids were recycled from the settler continuously. The aerobic biomass was replaced on day 57 as there had been a break in operation.

Biogas production and $\%CO_2$ were measured continuously using a gas meter (LFM 300, G H Zeal, London UK) and infra-red monitor (type SBG100-002-15290 ADC Ltd, Hodderson, UK). The pH of the UASB reactor and dissolved oxygen (controlled above 3 mg l^{-1}) and pH (controlled by addition of 1 M HCl) of the aerobic stage were monitored continuously.

Assay methods

The bicarbonate alkalinity (BA) of the UASB reactor was measured by titration to pH 5.75 (Jenkins *et al.*, 1983). The true colour, and hence colour removal, at each stage of treatment was determined by measuring the average OD of centrifuged samples at 436 nm, 525 nm and 620 nm in a manner similar to that described in British Standards (BS 6068, 1995) giving values in True Colour Units (TCU). The COD of samples was measured (APHA, 1989) with mercury-free reagents (HMSO, 1986). Each measurement was the mean of three replicates. BOD_5 was measured using the WTW OxiTop 1230 T system (Burmarc Ltd, UK) (total volume 250 cm^3, three replicates). Dilution water was made up as recommended by the Standing Committee of Analysts (1983) containing one capsule of polyseed per 500 ml (Hach Company, Germany). The MLSS of the aerobic stage was determined daily (APHA, 1989). VFAs were measured as described by Peck *et al.* (1986).

Experimental design

The rig (overall system HRT 1.8 days) was initially operated on a feed of 0.15 gl^{-1} dye and 1.9 gl^{-1} starch for 22 days (defined as Initial Experiment in Table 1) to obtain steady state values with good effluent quality. A programme of varying starch/dye content in the STE was then followed, operating for approximately 7 days at each new dye and starch concentration in three further experiments (Experiments 2–4 in Table 1) each followed by a return for 7 days to the Initial Experimental conditions (Experiment 1). Each Experiment 2–4 was repeated, thus Experiment 1 spanned seven separate 1-week periods and monitored the ability of the reactor to return to the Initial conditions after Experiments 2, 3 and 4. In Experiments 3 and 4 the starch was doubled to 3.8 gl^{-1} ("high starch"), and in Experiments 2 and 4 the dye was increased 5 fold to 0.75 gl^{-1} ("high dye"). There was an interval between day 51 and 52 when the system was not fed for 20 days. After day 130 there was a period of 26 days during which the reactor was not fed. A further experiment (Experiment 5) of 16 days duration after steady state conditions had been reached used 0.45 gl^{-1} dye ("medium dye") and 2.9 gl^{-1} starch ("medium starch").

During Experiment 5 a concentrated OECD synthetic sewage waste (OECD, 1981) was fed to the activated sludge stage at a rate of 1.4 l/day to simulate a textile effluent treatment plant where one third of the COD to the aerobic stage comes from domestic sewage. The OECD waste, stored in a refrigerator for 7 days maximum, contained 4.8 gl^{-1} of peptone, 3.3 gl^{-1} meat extract, 0.9 gl^{-1} urea, 0.21 gl^{-1} sodium chloride, 0.12 gl^{-1} calcium chloride dihydrate, 0.06 gl^{-1} magnesium sulphate heptahydrate, and 0.84 gl^{-1} potassium hydrogen phosphate.

RESULTS AND DISCUSSION

The TS and TVS in the UASB reactor at the start of this series of experiments were 33.7 gTS and 26.07 gTVS per litre of reactor. The maximum sludge loading rate (B_x) obtained in the UASB reactor used here was 0.15 kg COD kg^{-1} TVS d^{-1}. This was below the values achieved by An *et al.* (1996) of 0.269 kgCOD kg^{-1} TVS d^{-1} for UASB reactors fed with dye wastes and by Zhu *et al.* (1994) of 0.75 kg COD kg^{-1} TVS d^{-1} for UASB reactors fed with dye manufacturing waste at the end of the adaptation phase. However, the maximum volumetric loading rate (B_v) achieved in the UASB reactor used here was 3.9 kg COD m^{-3} d^{-1} (Experiment 4). This was apparently higher than the B_v of 2.8–3.12 kg COD m^{-3} d^{-1} achieved when treating dye wastes at a 10-h HRT by means of a UASB reactor (estimated from Zhu *et al.*, 1994) and lower than the 5.3 kg COD m^{-3} d^{-1} achieved by An *et al.* (1996).

Table 2. COD and BOD and percentage overall reduction by combined anaerobic-aerobic treatment system for all experiments[a]

Experiment	STE (mg l^{-1})			UASB effluent (mg l^{-1})			Final effluent (mg l^{-1})			Total % removal		
	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*
Initial (COD)	2287	285	12	877	130	12	477	92	9	79.3	5	9
Initial (BOD)	1068	29	3	377	66	3	14	10	3	98.7	–	3
1 (COD)	2222	132	7	750	218	7	441	269	7	80.3	11	7
1 (BOD)	1483	162	6	619	131	6	65	20	6	95.6	1	6
2 (COD)	2713	131	2	1303	153	2	911	245	2	66.4	12	2
2 (BOD)	1397	49	2	599	130	2	71	12	2	94.9	0.7	2
3 (COD)	3731	152	2	1462	229	2	444	16	2	88.1	0.9	2
3 (BOD)	2002	16	2	1151	348	2	102	13	2	94.9	0.7	2
4 (COD)	3902	83	2	1579	49	2	812	139	2	79.3	3.8	2
4 (BOD)	2550	281	2	1225	75	2	157	10	2	93.8	0.3	2
5 (COD)	3137	341	12	1205	333	12	723	104	11	77	3	11
5 (BOD)	1854	88	1	767	–	1	76	6	1	95.9	–	1

[a]*n* = number of repeats of experiments for Experiments 1–4, and number of daily readings for Initial and Experiment 5 once 3 HRT in the UASB reactor had elapsed. The mean was calculated for Experiments 1–4 from the average of each repeat.

Initial experiment and experiment 1

The results of the Initial Experiment and Experiment 1 (both low starch, low dye) were compared to ascertain whether the initial conditions were regained in between Experiments 2, 3 and 4.

The percentage reduction in COD by each stage (after 3 HRT) was similar for both sets of experiments, at 62–66% for the UASB reactor and 14–18% for the aerobic stage, giving an overall reduction of 79.3% and 80.3% for the Initial Experiment and Experiment 1, respectively. The overall percentage reduction in BOD by each stage was similar with about 65–58% reduction by the UASB reactor and 34–37% reduction by the aerobic stage giving an overall reduction in BOD of 98.7% and 95.6% for the Initial and first Experiment, respectively (Table 2).

True colour removal was poorer in Experiment 1, 56% compared with 73% in the Initial Experiment giving final colour readings of 0.41 and 0.28 TCU, respectively (Table 3). This could be due to adsorption occurring in the UASB reactor in the Initial Experiment, since for 52 days prior to this set of experiments the reactor had not been fed.

The UASB reactor effluent pH and BA were similar at 7.32 (SD = 0.14, $n = 13$) and 1766 mg $CaCO_3$ l^{-1} (SD = 95, $n = 13$) in the Initial Experiment compared with 7.28 (SD = 0.06, $n = 7$) and 1812 mg $CaCO_3$ l^{-1} (SD = 91, $n = 7$) in Experiment 1. Methane yields in Initial Experiment and Experiment 1 were both 0.18 l CH_4 g^{-1} COD added, although the CO_2 content was slightly different (25.6% and 26.9%, respectively).

The average MLSS was 3.30 gl^{-1} (SD = 1.17, $n = 11$) in the Initial Experiment compared to 1.91 gl^{-1} (SD = 1.5, $n = 7$) in Experiment 1. Change in MLSS was attributable to loss of biomass over time. The sludge loading rate (B_x) was 0.3–0.7 gBOD g^{-1} MLSS.d^{-1}, the latter value being rather high. Apart from MLSS and colour removal, performance during the Initial Experiment and Experiment 1 was similar. This indicated that differences observed in Experiments 2–5 were due to changes in experimental conditions and were not due to underlying changes in reactor microbiology.

Experiments varying dye and starch concentrations

COD and BOD. Table 2 shows the COD in STE, UASB reactor effluent and final effluent for each Experiment after 3 HRT. The percentage removal from each stage for Experiments 1–5 after 3 HRT can be seen in Figure 1. Most COD removal occurred in the UASB reactor. At high starch concentrations (Experiments 3 and 4) % COD reduction in the UASB reactor was similar at both dye concentrations (60–61%). The lowest final effluent COD (~440 mg l^{-1}) was obtained at 0.15 g l^{-1}

Table 3. True colour and percentage overall reduction for all Experiments[a]

	True colour STE (TCU)			True colour UASB effluent (TCU)			True colour final effluent (TCU)			Total % removal		
	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*	mean	SD	*n*
Initial	1.01	007	12	0.42	0.07	12	0.28	0.08	9	72.5	6.5	9
1	0.93	0.07	7	0.55	0.07	7	0.41	0.09	7	55.6	9.9	7
2	5.05	0.11	2	3.12	0.26	2	2.76	0.44	2	45.1	9.8	2
3	0.92	0.04	2	0.38	0.01	2	0.21	0.01	2	77.1	0.02	2
4	4.3	0.13	2	1.9	0.12	2	1.4	0.01	2	68.3	1.3	2
5	2.38	0.33	9	1.06	0.11	9	0.76	0.25	8	67.2	13.6	8

[a]Note: *n* = number of repeats of experiments for Experiments 1–4, and number of daily readings for Initial and Experiment 5. The mean was calculated for Experiments 1–4 from the average of each repeat.

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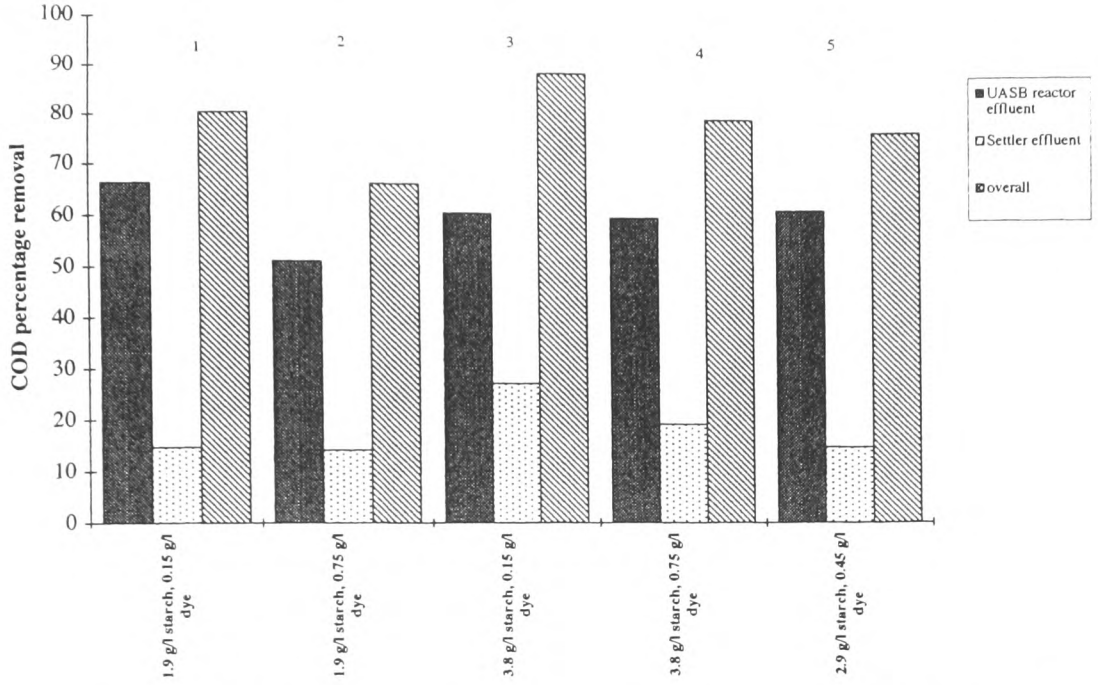

Fig. 1. Percentage COD removal by the UASB reactor, aerobic stage and overall for each Experiment at steady state.

dye with both low and high starch concentrations. The poorest overall COD reduction (66.4%) was obtained in Experiment 2, where the contribution of the dye to the COD was highest. In Experiment 5 the COD of the final effluent was between the values obtained at 0.15 gl^{-1} dye and 0.75 gl^{-1} dye (Table 2), indicating that COD removal was affected by dye COD. The highest overall percentage COD removal (88.1%) was obtained in Experiment 3, which also showed the greatest contribution to COD removal by the aerobic stage (Fig. 1). The highest B_x to the UASB reactor (Experiment 4) was 0.15 kg COD kg^{-1} TVS d^{-1}. For Experiment 5 B_x was 0.11 kg COD kg^{-1} TVS d^{-1}.

The overall COD removal in all experiments, with the exception of Experiment 2, was similar to or greater than the values obtained by Zaoyan *et al.* (1992) and Loyd *et al.* (1992). Zaoyan *et al.* found that 78% COD and over 95% BOD removal were achieved when dye wastewater was treated by means of anaerobic–aerobic methods. Basibuyuk and Forster (1997) showed 80% COD removal by an anaerobic filter using an STE with two dyes, and 92% removal after an aerobic stage. The final effluent COD in the Initial Experiment and Experiments 1 and 3 was between 440 and 480 mg l^{-1}. A fixed emission standard of 125 mg l^{-1} COD is required in the UK under the Urban Wastewater Treatment Directive for populations > 2000 (Gray, 1999).

Figure 2 illustrates the percentage reduction of BOD by each stage. With the exception of the Initial Experiment, the final BOD values were quite high (65–157 mg l^{-1}, see Table 2) compared to the Royal Commission Standards for discharge (20 mg BOD l^{-1}) showing that tertiary treatment would be needed before final discharge. The highest final BOD values were obtained at high starch concentration, with Experiment 4 (0.75 g l^{-1} dye) being higher than Experiment 3. A reduction in the BOD value of 94–95% was achieved in each case for Experiments 1–5, similar to that achieved by Zaoyan *et al.* (1992) and exceeding that of Loyd *et al.* (1992). It should be noted that the performance reported here was obtained without waiting three sludge ages for adaptation of the aerobic sludge.

An *et al.* (1996) found dye manufacture wastewater to be biodegradable at BOD:COD of above 0.25. The BOD:COD of the STE exceeded this ratio for every experiment in this study. The BOD:COD ratios for STE and UASB reactor effluent increased considerably in Experiments 1, 3 and 4 (up to 47%, Table 2) indicating that the components had become more amenable to degradation, presumably by a change of the dye's molecular structure.

VFA concentrations in the UASB reactor. In Experiments 1, 2 and 5 the mean VFA concentration was near to, or below, 300 mg l^{-1}, the maximum concentration recommended by Carliell *et al.* (1996), indicating that the reactor was not stressed. Propionic acid levels were 25–80 mg l^{-1}. In Experiments 3 and 4 at high starch concentration, the reactor contained higher VFA concentrations (a mean TVFA of 960 and 730 mg l^{-1}, respectively) including higher concentrations of propionic acid

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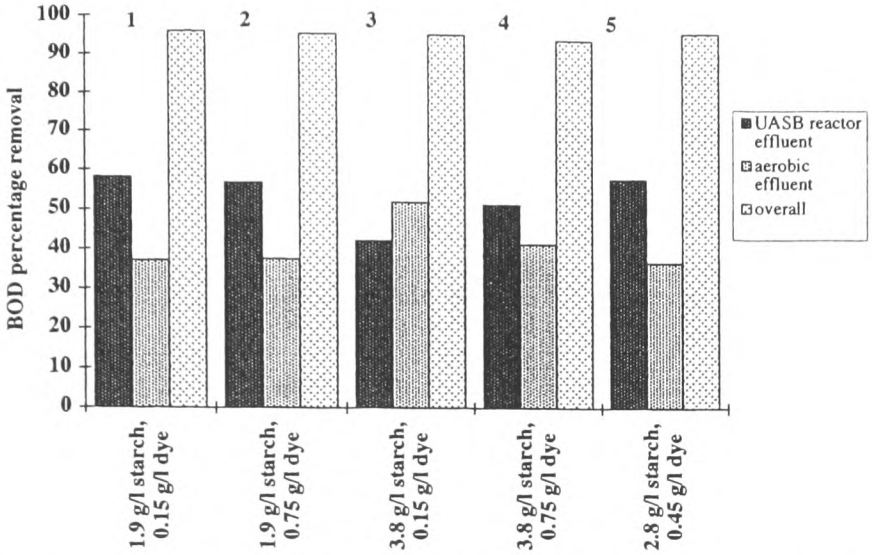

Fig. 2. Percentage BOD removal by the UASB reactor, aerobic stage and overall after steady state operation had been achieved.

(180 and 120 mg l^{-1}, respectively), suggesting instability. The medium starch feed (2.9 gl^{-1} Experiment 5) did not cause an increase in VFA.

The pH of the STE increased from pH 9.6 at low starch concentration to pH 10.8 at high starch concentration. Dye concentration had little effect on STE pH. The pH of the UASB reactor was similar in Experiments 3 and 4 at pH 7.2, compared to pH 7.3 in Experiments 1, 2, and 5. The bicarbonate alkalinity of the UASB reactor increased from 1812 and 1786 mg l^{-1} in Experiments 1 and 2 to 1972 and 2054 mg l^{-1} in Experiments 3 and 4, respectively, despite a rise in VFA concentrations. High starch concentrations gave a concomitant rise in NaOH used in hydrolysis, providing more buffering capacity as $NaHCO_3$. In Experiments 3 and 4 the VFA:BA ratio was higher than 0.3 and the system was deemed to be unstable (Carliell *et al.*, 1996). It is therefore apparent that 3.8 gl^{-1} starch at a 1 d HRT was the maximum load that could be tolerated by the UASB reactor in this study.

Colour Removal. Table 3 shows the mean true colour determined at each treatment stage for Experiments 1–5. A maximum overall colour removal of 77% was achieved in Experiment 3, giving the lowest final value (0.21 TCU). Colour removal achieved in Experiments 3–5 was in excess of this value and closer to the 71.6% removal achieved by Zaoyan *et al.* (1992). It was within the range reported by Carliell *et al.* (1994) and Boe *et al.* (1993) but below that achieved by An *et al.* (1996) and Loyd *et al.* (1992). The anaerobic colour removal in Experiment 5 (more starch but less dye than Experiment 2), was 55%. Colour removal by the UASB reactor was similar in Experiment 3 (59%) and Experiment 4 (57%) despite different dye concentrations in these experiments. The higher percentage colour removal in Experiments 3 and 4 (high starch) compared with Experiments 1 and 2 (low starch) indicates that starch assists colour removal. The absorbance of the final effluent at 525 nm was at best around 0.3 absorbance units (Experiment 3). Colour consents for sewage treatment works in the Severn Trent area (Churchley, 1994) at 550 nm were between 0.012 and 0.025 while a typical consent from the UK Environment Agency (Shewsbury) at 550 nm is 0.055 (Pers. Comm.). Further physico-chemical treatment for colour removal is thus required.

In Figure 3 the percentage overall colour removal and dye concentration in the STE is plotted against the starch:dye ratio (12.7, 2.53, 25.3, 5.07, and 6.4 for Experiments 1–5, respectively). At identical dye concentrations (comparing Experiments 2 and 4, high dye, and Experiments 1 and 3, low dye) the presence of extra starch greatly increased colour removal. The extra starch may be providing an increased source of reducing equivalents for azo bond cleavage, enabling more dye to be degraded (Carliell *et al.*, 1996).

Gas composition and flow rate. The methane yields after 3 HRT in Experiments 1 and 2 were 0.18 and 0.15 l CH_4 g^{-1} COD added and in Experiments 3 and 4 were 0.15 and 0.18 l CH_4 g^{-1} COD added, respectively, indicating that dye concentration in the range tested did not inhibit methanogenesis in the anaerobic stage. The mean $\%CO_2$ at steady state during Experiments 1–5 was 26.9, 25.8, 29.6, 29.3 and 26.1%, respectively. Experiments 3 and 4 had higher $\%CO_2$ in the biogas than Experiments 1 and 2 and higher levels of VFA suggesting the UASB reactor was less stable.

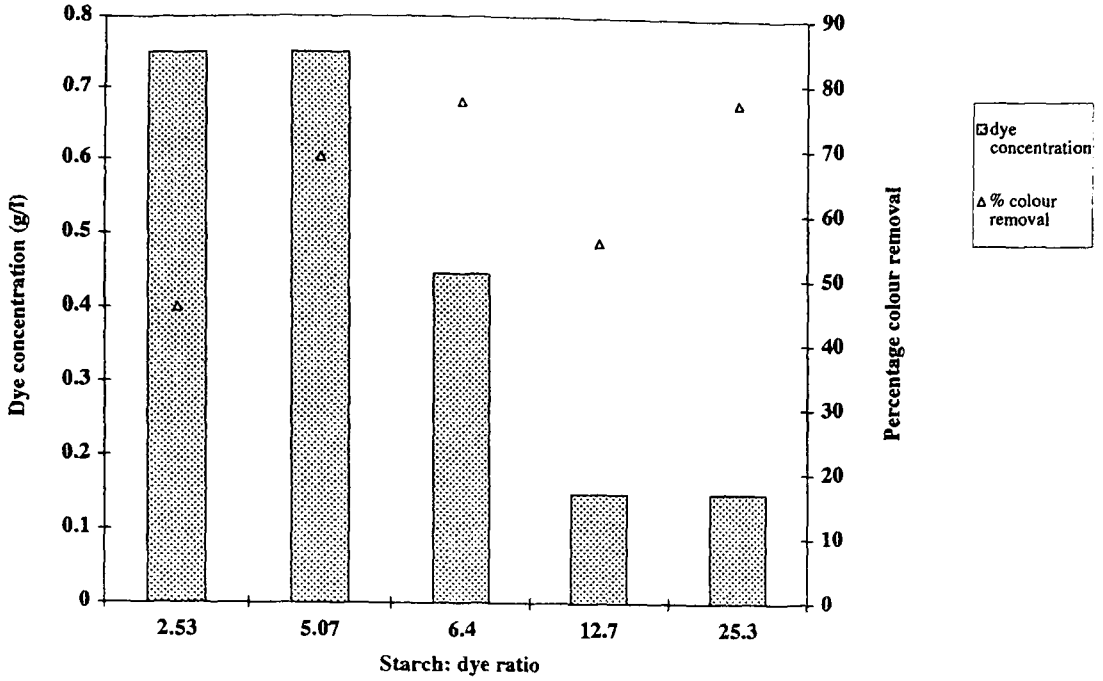

Fig. 3. Percentage colour removal by UASB reactors and dye concentration in STE vs the starch:dye ratio.

MLSS. The sludge replaced on day 57 was not acclimatised to the UASB reactor effluent. However the MLSS remained high at approximately 4 gl^{-1} during the next 14 days (7 days at the condition of Experiment 1 and 7 days at Experiment 3). The MLSS then decreased over the next 21 days, (conditions of Experiments 1 and 2), to approximately 1 gl^{-1}. Over the next 7 weeks of experiments, at high starch (Experiments 3 and 4) MLSS and the rate of oxygen consumption increased, and at low starch (Experiment 1), MLSS fell, giving a B_x much higher than for a well performing activated sludge plant. These effects suggested that biomass growth in the activated sludge stage was limited by carbon source. When the activated sludge stage was supplemented with OECD synthetic sewage (Experiment 5) the MLSS concentration was maintained around 2.43 gl^{-1} (SD = 0.92, n = 12) giving B_x 0.76 gBOD g^{-1} MLSS d^{-1}. It must be noted that to achieve true steady state in the aerobic as well as the anaerobic stage it would have been necessary to operate for more than three sludge ages (at least 15 days) at each experimental condition, more than doubling the duration of the experimental programme.

CONCLUSIONS

The treatment efficiency of the system (Experiment 1 conditions, 1.9 gl^{-1} starch and 0.15 gl^{-1} dye) remained constant over 130 days despite seven intervening 1-week periods of operation at different starch:dye ratios. This indicates that the system tolerated step changes without any change in effectiveness of operation.

The UASB reactor improved the treatability of the effluent, the BOD:COD ratio rising by up to 47% compared with the STE feed.

The aerobic stage compensated for the lower percentage COD removal by the UASB reactor when the loading rates were high.

The maximum BOD removal was 99% and the maximum COD removal was 88% giving a final COD of 444 mg l^{-1} in the system effluent.

Addition of synthetic sewage to the aerobic reactor was required to maintain MLSS levels at a low organic loading rate.

A maximum of 77% overall colour removal was achieved at high starch and dye concentrations in the feed (3.8 and 0.15 gl^{-1}). Most colour removal occurred anaerobically.

At both low (0.15 gl^{-1}) and high (0.75 gl^{-1}) dye concentration a starch concentration of 3.8 gl^{-1} gave a better colour removal than a starch concentration of 1.9 gl^{-1}, however the UASB reactor began to show signs of instability at the higher starch concentration. As medium starch concentration (2.9 gl^{-1}) was tolerated by the system, the maximum starch concentration for a stable system was considered to fall between 2.9 and 3.8 gl^{-1}.

The optimum starch:dye ratio for overall colour removal varied with the initial dye concentration used.

It is recommended that if colour removal efficiency is seen to decrease, then carbohydrate should

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be added to the feed at a maximum B_x to the anaerobic stage between 0.11 and 0.15 kg COD kg^{-1} TVS d^{-1}.

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